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POLYGONUM: SECTIO TOVARA

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(WITH PLATES I, II)

It is often interesting to follow the history of some genus from the earliest works on botany until recent time. So far as concerns *Polygonum*, several species were known as far back as the time of DIOSCORIDES, and it is really remarkable to see how some of the oldest names have been preserved, being applied to sections or subgenera. The earliest attempts to define the species depended upon the mere habit of these plants, and sometimes their properties. The name *Polygonon* was used by DIOSCORIDES for *Polygonum maritimum* and *P. persicaria* var. *incanum; Hydropiper* for *P. hydropiper;* and *Helxine* for *P. dumetorum*. Our *Polygonum convolvulus* was called *P. hederaceum* by FABIUS COLUMNA, while DODONAEUS and LOBELIUS had it under the name *Helxine cissampelos*. *P. bistorta* is BRUNFELS' *bistorta; P. aviculare* was called *Centumnodia* by BRUNFELS, *Sanguinaria* by LOBELIUS "a cohibendo sanguine," and this is the only species of the genus which JOHANNES BAUHIN (3) has enumerated as being named for a saint, "S. Innocentii herba." The name *Polygonum*, however, was also used by the early writers for several other plants of the same habit, for instance *Radiola*, *Lepigonum*, *Herniaria*, etc., according to CASPAR BAUHIN (2). While the genus had thus been elaborated to some extent by writers before BAUHIN, this author deserves credit for being the first to present a systematic arrangement of the species with generic names: *Bistorta*, *Persicaria*, *Hydropiper*, *Polygonum*, and *Convolvulus* ("minor semine

triangulo"). He was the first writer to use binomials, and his work, containing a large number of synonyms, dating back to the earliest writers, represents the most important contribution to scientific botany of that period.

The arrangement of the genera by BAUHIN was of course anything but natural from a modern point of view. The habit of the plants and sometimes their properties were considered the most important. Consequently the genera described by BAUHIN are frequently widely separated, as may be seen from *Polygonum. Hydropiper*, for instance, is placed among acrid herbs (2) such as Cruciferae and Piperaceae. *Bistorta* is placed near *Statice* and *Aroideae*, evidently on account of the large tuberous rhizomes. *Polygonum*, our *P. aviculare*, heads a group of plants among which are *Sedum*, *Sempervivum*, and *Saxifraga*, several of which have small crowded leaves. Finally his *Convolvulus*, which comprises the true genus and our *P. convolvulus*, is in the same chapter as *Smilax*, *Bryonia*, *Lupulus*, and *Vitis*. An alphabetical arrangement of the genera might have been more appropriate, but BAUHIN preferred to arrange the plants in accordance with his idea of natural affinities, and his system is indeed far superior to those proposed by his predecessors. In the course of the century that elapsed between the works of BAUHIN and TOURNEFORT (24) several new species of *Polygonum* were described, notably in the writings of PARKINSON (1640), MORRISON (1680), RAY (1686), and PLUKENET (1696), and among these species was our *Polygonum virginianum: "Persicaria* frutescens maculosa, Virginiana, flore albo" Parkinson, subsequently described in the same manner by RAY. Contrary to BAUHIN, who named only the genera but described the species, TOURNEFORT described the genera, enumerating the species with the diagnoses given by the respective authors, without appending any diagnoses himself. His system was based upon the floral structure, and carried through with remarkable care. The plants were arranged in classes, with sections comprising the genera and species, accompanied by numerous figures. His classification of the genera, however, was in many cases anything but natural. TOURNEFORT never made any use of the works of RAY, who drew the distinction between Cryptogames and Phanerogames, and who established the two groups Monocotyledones and Dicotyledones. The the-

ory proposed by CAMERARIUS was not accepted by TOURNEFORT. Nevertheless some genera, of which the floral structure is very uniform, were arranged in the same section, as for instance the *Polygonum* alliance. This is described in the 15th class, "flore apetalo seu stamineo," together with *Rumex*, several Chenopodiaceae, besides *Asarum*, *Herniaria*, *Paronychia*, *Alchemilla*, and some others. At the end of the same class we find the Gramineae, Cyperaceae, *Ricinus*, *Hippuris*, and some Urticaceae. TOURNEFORT described *Persicaria*, *Polygonum*, *Fagopyrum*, and *Bistorta* as genera, but he referred *Helxine* (*Polygonum convolvulus*) to *Fagopyrum*, together with *F. scandens americanum* (Hermannus), known now as *Polygonum scandens*. The floral structure being so very uniform, TOURNEFORT inserted also some characters taken from the habit, for instance: "flores in foliorum alis nasci" (*Polygonum*); "spica" and "radices tuberosae" (*Bistorta*); "occurrunt etiam species quibus praeter flores et semina innascuntur tubercula" (*Bistorta alpina minor* Bauhin), which is our *P. viviparum*. It would be very difficult, however, to determine the species of his *Persicaria; P. virginianum*, for instance, appears only with the brief diagnosis of PARKINSON, and may hardly be distinguished from the numerous other Persicariae.

These various systems were intended for demonstrating the natural relationship of the plants by means of some arrangement of the genera. From a practical viewpoint, however, the systems were a failure, since they did not facilitate the determination of the plants, and especially not the genera. Some other kind of system was needed, one that would make the knowledge of plants more accessible. The Linnaean system, artificial as it was, facilitated the determination of plants, genera as well as species. The method to be followed when using this system is so simple that it was illustrated by only 24 small figures on one plate (Genera plantarum, ed. 2, Leyden 1742). The only difficulty in using this system is the fact that there are several genera of which the number of stamens is not the same in all the species of the same genus. In *Polygonum*, for instance, a member of the eight class, all the species are placed together, even though the number of stamens fluctuates between five and eight. This is pointed out by LINNAEUS himself, where under *Persicaria* the statement is made: "Stamina et pistillum quoad numerum in hoc genere, incerta

sunt." In his earlier years LINNAEUS (13) regarded *Persicaria*, *Bistorta*, *Polygonum*, and *Helxine* as genera. There was only one species of *Polygonum*, *P. aviculare; Helxine* comprised *Polygonum convolvulus* and its allies, besides *P. arifolium* and *P. sagittatum*. *P. virginianum* was a member of *Persicaria*, but with the diagnosis much improved: "florum staminibus quinis, stylo duplici, corolla quadrifida inaequali." In this same work we find the earliest mention made of the nectaries, namely in *P. orientale:* "Glandulae nectariferae manifestae tot sunt, quot stamina, uti in *Helxine* videre est." The six species of *Persicaria* enumerated are so well described, with a very few words, that their identification is very easy; for instance, *P. lapathifolium:* "staminibus quinis corollae regulari aequantibus"; and *P. amphibium:* "staminibus quinis corollae superantibus," etc. These genera were later referred to *Polygonum*, but the species, 27 in all, were arranged in the same manner as before under the respective sections *Bistorta*, *Persicaria*, etc. In describing *P. scandens* LINNAEUS mentions a pore at the base of the petiole, a structure which seems to be very rare in the genus, and described only as characteristic of *P. cilinode* Michx. in the monograph by MEISNER (14). Thus, while LINNAEUS adopted the classification of *P. virginianum* as proposed by TOURNEFORT and his predecessors, namely, as a *Persicaria*, ADANSON (1) established the genus *Tovara*, based upon this species.

ADANSON named the family Persicariae, containing the genera *Tovara*, *Persicaria* Tour., *Polygonum* Tour., *Fagopyrum* Tour., *Helxine* L., and *Bistorta* Tour. The generic characters of *Tovara* were given as follows: "épilâche terminale, calice 4 divisions, étamines 5, stigmates 2 cylindriques, graine lenticulaire." With reference to the nectaries, this author observed them in species of *Persicaria* and *Fagopyrum*. With the exception of the inflorescence, a loose flowered spike, the other characters are about the same as those LINNAEUS mentioned in his diagnosis. ADANSON, however, did not notice that the lobes of the perianth are of unequal length, as correctly described by LINNAEUS. The very little that was actually known about *Tovara*, and evidently the lack of complete material, especially of the fruiting stage, must have been the reason why it became suppressed for so many years. Even MEISNER would not recognize *Tovara*, even as a mere section. The genus *Polygonum* L. was by this author di-

vided into seven sections: *Bistorta, Fagopyrum, Persicaria, Avicularia* (sectio *Polygonum* L. partim), *Tiniaria* (sectio *Helxine* L. partim), *Aconogonon* Meisn., and *Amblygonon* Meisn. Our *Polygonum virginianum* appears here as a member of *Persicaria,* and very little of importance is added to the diagnosis as given by LINNAEUS and ADANSON, while the figures on plate III and the explanation of these contain several new points: the diagram of the flower, the nectaries, the pistil with the two long styles slightly diverging at the apex and reflexed at maturity of the fruit, all being characters of importance, and sufficient for accepting *Tovara* as a section; and these figures were drawn by MEISNER himself.

In the works of TORREY (23) and GRAY (8) we find *Tovara* described as a monotypic section, and TORREY has given an excellent diagnosis comprising the most important sectional characteristics, among which may be cited the very long virgate spike, with one or two flowers from each bract; the unequally four-parted calyx (perianth); the five somewhat unequal stamens; the rigid, parallel styles exserted when in fruit, bent obliquely downward at the base so as to form an obtuse angle with the ovary, the summits recurved; stigmas minute; achenium large, strongly curved on both sides, beaked with the persistent styles; embryo curved against one of the edges of the albumen, the cotyledons oblong. TORREY, however, does not mention the nectaries within this or in any of the other sections; the habit is given as perennial only. The description given of *Tovara* in GRAY's manuals agrees with the one by TORREY, and even in the last edition (1908) the nectaries have been overlooked, and no additional statement is made as to the vegetative reproduction.

In BENTHAM and HOOKER's *Genera plantarum* (1883) *Tovara* is described as a section, with *Antenoron* Rafin. as a synonym. Two species are said to be known, one in North America, another in Japan, but closely related to each other. Ten sections are recognized by BENTHAM and HOOKER, among which *Avicularia, Persicaria, Cephalophilon* (*P. sagittatum, P. arifolium*), *Tiniaria, Pseudopolygonella,* and *Tovara* are represented in North America. Furthermore, DAMMER (7) accepts *Tovara* as a section, but gives only a rather incomplete diagnosis as compared with the one by TORREY. According to DAMMER the Japanese species is *Polygonum filiforme* Thunbg., which

by MEISNER is not placed very close to *P. virginianum*, nor does the brief diagnosis indicate any close relationship. THUNBERG (22), however, gives a more comprehensive description of the species (*P. filiforme*), mentioning the four-parted perianth, the five stamens, the two styles being as long as the filaments of the stamens. The spikes are said to be filiform, and the leaves ovate with the ochreae ciliate, characters that might indicate identity with *Tovara*, at least at the stage of flowering, but the important fruiting stage is not included in the diagnosis. ADANSON mentions that a species of *Tovara* grows in China, from which a blue dye is obtained; this species, however, may hardly be *P. filiforme*, since THUNBERG only mentions *P. chinense* L., *P. barbatum* L., and *P. aviculare* L. as being frequently cultivated for that purpose.

Finally, BRITTON and BROWN (4) have not referred other species than *P. aviculare* and its nearest allies to *Polygonum* as a genus, raising the sections *Tovara, Persicaria, Bistorta, Tracaulon* (*P. sagittatum*), and *Tiniaria* to generic rank. Of these *Tovara* is described as annual or perennial herbaceous; the two styles are said to be recurved or curled, and the figure shows the fruit with the styles diverging from the base and reflexed, errors that would have been avoided if the authors had examined the living plant and compared the diagnosis given by TORREY. The floral nectaries are not mentioned by these authors, and the characteristic extra-floral nectary in *Polygonum cilinode* and *P. scandens* has also been overlooked.

The classification adopted by BENTHAM and HOOKER, keeping *Polygonum* intact but distinct from *Fagopyrum*, appears the most natural. It is a polymorphous genus as to habit and floral structure, but the sectional distinctions are not sufficient for the establishment of separate genera, as has been done by BRITTON and BROWN. For instance, the four-parted perianth, the number of stamens fluctuating between four and six, and the two styles are not characteristic of *Tovara* alone, for these structures recur in *Tephis* Adans., a section closely related to *Avicularia*, and consequently with a habit widely different from *Tovara*. A synopsis of the sections, showing these various structures, is given by MEISNER, but, as already stated, this author did not recognize *Tovara* as a section, placing the species as a

member of *Persicaria*. Moreover, the name *Helxine* is changed to *Tiniaria*, which according to SPRENGEL (21) was proposed by MARCEL DE BORDEAUX for *Polygonum convolvulus*.

We have thus in the genus *Polygonum* an example of very old systematic botany, where a genus has been preserved, and where some of the species have been understood in a very natural manner, corresponding with our modern viewpoint. *Polygonon* of DIOSCORIDES comprises the sections *Avicularia* and *Persicaria;* his *Helxine* corresponds with the section of the same name by LINNAEUS, but afterwards changed to *Tiniaria;* the *Bistorta* of BRUNFELS is our subsection *Bistorta*. It is also interesting to see that FABIUS COLUMNA regarded *Helxine* as a *Polygonum*, calling it *P. hederaceum*, a case of generic synonymy. According to LINNAEUS all these were genera, but only for some time, his final classification reducing them to one genus, "*Polygonum*," with sections. With the exception of *Fagopyrum*, which is not included in *Polygonum* by BENTHAM and HOOKER, the other species are still regarded as members of the genus; however, with some modifications of the sections. For instance, *P. sagittatum* and *P. arifolium* are removed from *Tiniaria* and referred to *Cephalophilon; Bistorta* is removed to *Persicaria; Tovara* is established, etc.

While the more recent classification of the species of *Polygonum* is based principally upon the floral structure, the general habit has also been considered, but to a much less extent. From this particular viewpoint the genus is not very interesting, judging from the relatively scant information obtained from the literature, and the manuals seldom contain other statements than "annual or perennial." "Ours all herbaceous with fibrous roots except in *P. viviparum*" is the generic characterization as given in the seventh edition of GRAY'S manual, and "a bulb-like caudex" is attributed to the section *Bistorta*. The subterranean stem structure of some of these has been described carefully and explained many years ago; for instance, *Polygonum amphibium* (10), *P. bistorta*, and *P. viviparum* (17), and ought to have been considered. With reference to the section *Tovara*, *Polygonum virginianum* is not well known from a morphological point of view, and the following notes may serve as a contribution to the knowledge of this rather singular species. Being an inhabitant of

shady woods, I have for the sake of comparison included the anatomical structure of some species of the sections *Persicaria*, *Avicularia*, and *Cephalophilon*, representing heliophilous types.

SUBTERRANEAN STEM.—MEISNER has figured the seedling stage. The cotyledons are epigeic, elliptic; there is a distinct hypocotyl, and the primary root is relatively long, slender, and ramified. I have not seen the stages intermediate between the seedling and the mature plant (fig. 1), but judging from the structure of the subterranean stem, which in several respects agrees with that of *Polygonum amphibium* (10), it seems probable that the first stolons are developed from cotyledonary buds common to several species of the genus. Fig. 1 shows a horizontally creeping, subterranean stem terminated by an erect floral shoot (*St*). Four stolons are developed, densely covered with tubular bladeless leaves, but with no roots. Secondary roots, on the other hand, are well represented upon the old stem internodes; they are thin, amply ramified, and proceed from the nodes or between these. The old stem is two years old, and considerably thicker than the stolons, which develop into aerial (mostly floral) shoots in their second year of growth. The mother stem does not persist, and withers gradually. The vegetative reproduction thus enables the plant to become distributed and to form new individuals. "Perennial by stolons" would be the proper characterization of this species, instead of simply "perennial," and I have never found any specimens that were annual.

FLOWER (figs. 2–5).—All the flowers are lateral, arranged in a long, thin, spicate inflorescence, and the subtending leaves are merely represented by ochreae, very remote from one another. Each of these ochreae subtends a three-flowered, secondary inflorescence of the rhipidium type, where the apparently central flower is the youngest.

As regards the diagram of the flower (fig. 2), the lobes of the perianth are exactly opposite: two outer and two inner ones, but there are apparently six stamens. Of these the four outer ones actually represent only two duplicated, which explains their position, while the two inner ones are exactly opposite the inner lobes of the perianth. The floral diagram of *P. virginianum*, as shown in fig. 2, thus corresponds with that of *Oxyria digyna* according to EICHLER (6),

with the exception, however, that in our *Polygonum* the arrangement of the floral organs is tangential instead of radial to the main axis. Moreover, in *P. virginianum* the anthers of the outer stamens are introrse, those of the inner ones extrorse, while in *Oxyria* all the anthers are introrse. This diagram of *Polygonum* recurs in many other species of the genus, according to EICHLER. It is not the only type of diagram which we have observed, however, since many flowers have only five stamens, the posterior being undeveloped; in the last flower to open we noticed almost constantly six stamens. Nectaries in the shape of small glands (fig. 4) alternate with the stamens. The perianth is somewhat unequally four-parted, the two outer lobes being a little shorter than the inner ones; the two inner stamens are a little longer than the others. At the time of pollination all the anthers are open, and the two styles are somewhat shorter than the stamens, although with the minute stigmata viscid, capable of receiving the pollen. After pollination has been effected the parallel styles increase considerably in length (fig. 5), become rigid and deflexed, forming an obtuse angle with the ovary, and with the summits recurved, bearing the minute stigmata. The perianth persists, tightly inclosing the achenium, and falls off at maturity.

According to MUELLER (15) the flowers of some species of *Polygonum* are adapted to pollination by insects, for instance *P. bistorta*, in which the stamens at the time of flowering are distinctly longer than the perianth, while the styles do not attain that length before the stamens have withered completely and dropped off (proterandrous dichogamy). In *P. viviparum* the flowers vary from bisexual, proterandrous, to purely female, but in *P. persicaria* and *P. lapathifolium* the pollination fluctuates between self and cross-pollination, since the flowers are bisexual, relatively small, and secrete very little nectar. Although the flowers of *P. minus* Huds. are not smaller than those of *P. persicaria*, they are nevertheless much more seldom visited by insects, owing to the loose, thin inflorescence, making the flowers less conspicuous than in the dense flowered spikes of *P. persicaria*.

In *P. virginianum* the flowers are constructed in about the same manner as those of *P. persicaria* and *P. minus*, thus being adapted to self or cross-pollination. Self-pollination seems undoubtedly to be

the more frequent, since the flowers are very inconspicuous, being small, and the inflorescence very lax. The three-flowered rhipidia are very remote, and there is almost a month between the opening of the first and the last developed flowers. No visitors were observed, nevertheless mature fruits were found in many specimens. The singular structure of the two persistent, rigid, hooked styles facilitates the dispersal of the fruit by means of animals, and at the same time the stoloniferous habit enables the plant to spread over a larger area. The species seems to be very particular with regard to the nature of environment; however, I have never found it outside shady woods. Some specimens, transplanted to the open along a creek, failed to produce flowers and lived only one year.

INTERNAL STRUCTURE OF VEGETATIVE ORGANS.—As stated, an account will be given of the anatomical structure of the section *Tovara*, as represented on this continent, besides some species of the sections *Persicaria*, *Avicularia*, and *Cephalophilon*, in order to show the probable correlation between structure and environment.

Polygonum virginianum L.—The roots are of the nutritive type, being very slender and of relatively short duration. Secondary tissues appear early, but are confined to the development of a narrow zone of secondary cortex surrounding a stele, of which the greater portion is occupied by numerous strata of thick walled conjunctive tissue, arranged very regularly in radii and extending to the center. The stele is frequently heptarch, but with only a few wide vessels in each ray. No cork was observed, and the endodermis as well as the primary cortex persists for some time, but more or less collapsed.

The stolons, at the stage of those in fig. 1, are cylindrical and glabrous; the cuticle is thin, smooth, and the outer cell wall of the epidermis is slightly thickened. The cortex consists of 8–10 compact, homogeneous strata, filled with starch, and the hypodermal layer becomes a phellogen. There is no endodermis, but an almost closed sheath of stereome, about three layers and moderately thick walled. The stele contains a single band of many collateral mestome strands, and between these an interfascicular cambium has developed some few strata of libriform, but of libriform only, since no purely leptomatic strands appear in the subterranean stem portions. The libriform forms a closed sheath between the inner flank of the hadrome

and the periphery of the pith, which is compact, solid, not hollow, filled with starch, and some cells contain a brown gelatinous substance.

By the time the stolons are in their second year of growth and have produced an aerial shoot, they have increased considerably in thickness, corresponding with the thick stem portion from which they have developed (fig. 1). The peripheral tissue is now cork, relatively thick walled, and in five or six strata, surrounding the primary cortex and stereomatic pericycle. Inside of this a secondary cortical parenchyma has developed, consisting of about ten strata, filled with starch, and several cells containing a brown gelatinous substance, as observed in the stolons. The stele now shows a band of separate leptome strands in the same radii as the stereome strands of the pericycle, several strata of cambium, some deep, but very narrow rays of hadrome and secondary libriform. The interfascicular tissue is represented by broad rays of starch bearing parenchyma extending from the pith to the secondary cortex. The pith is compact, thin walled, and contains much starch, and the gelatinous brown substance is visible in many cells. The increase in thickness has thus been effected by the activity of the intrafascicular and interfascicular cambium developing a secondary cortex, secondary mestome, and libriform and medullary rays at the expense of most of the interfascicular libriform, which has become more or less obliterated. Also, the formerly closed pericycle has become divided into separate arches of stereome.

In the aerial stem the internodes between the large green leaves are cylindrical and glabrous. The cuticle and the epidermis show the same structure as in the stolons, but no phellogen appears; no stomata were observed. Collenchyma is well represented as a continuous, hypodermal zone of about four strata surrounding the cortex proper, which consists of four or five layers of chlorophyll-bearing parenchyma, with relatively wide, rhombic intercellular spaces. Several of the cells contain aggregated crystals of calcium oxalate, and the brown gelatinous substance mentioned was also noticed in some of the cells. There is a large celled endodermis, of which several cells have become differentiated into long tubular reservoirs containing tannin of a clear, brownish color. A closed sheath of thick walled

stereome in three or four strata surrounds a stele of a single band of separate, collateral mestome strands alternating with some very thin strands of pure leptome (fig. 13). Between these mestome strands are layers of libriform developed from the interfascicular cambium. The pith is thin walled, not hollow, and contains starch and aggregated crystals of calcium oxalate. Tannin reservoirs of great length are scattered in the pith (figs. 11, 12).

A somewhat modified structure is to be observed in the swollen nodes. In these the complete cortex is collenchymatic, about fifteen layers, and very narrow in proportion to the large pith. The endodermis is thin walled, and a few of the cells are tannin reservoirs. There is no stereomatic pericycle, the mestome strands bordering directly on the endodermis. The structure of the mestome is as described, but the libriform (inter as well as intrafascicular) is very thin walled. Purely leptomatic strands are much less frequent than in the internodes. The pith is thin walled, and contains numerous aggregated crystals of calcium oxalate, besides tannin.

The very thin internodes of the inflorescence are cylindrical and glabrous; the epidermis is thick walled, almost collenchymatic; and the cortex consists of about ten strata of parenchyma with a little chlorophyll. There is no endodermis, but a closed sheath of thick walled stereome in six or seven strata surrounds a band of many collateral mestome strands alternating with some which are purely leptomatic. The narrow pith is thin walled, starch-bearing, and contains also tannin.

In comparing the structure of the subterranean internodes with the aerial, the development of secondary tissues in the former naturally results in cork and a secondary cortex in the subterranean, while in the aerial internodes the growth of the inner tissues does not interfere with the preservation of the peripheral tissues. While the mechanical tissue, the stereome, occurs as a sheath around the mestome strands in both stems, the collenchyma is confined to the aerial, representing the whole cortex in the nodes. Another difference depends upon the non-development of purely leptomatic strands in the subterranean internodes, besides the absence of tannin reservoirs.

The structure of these internodes, subterranean and aerial, thus illustrates a case where some of COSTANTIN'S (5) conclusions cannot

be accepted, at least not as a general rule. According to this author the subterranean stem should be destitute of stereome, in the form of a pericycle, and as strands encircling the hadrome. The cambium, intra and interfascicular, should be less active, and the pith should be narrower than the cortical parenchyma. As already described, stereome is well represented in the subterranean stem; the cambium is active by developing a secondary cortex and secondary libriform; and the difference in width of the pith is but very slight. On the other hand, the development of cork, the disappearance of the collenchyma, and the increase in width of the cortical parenchyma are structures characteristic of the subterranean stem of our *Polygonum*, and are in accordance with the observations made by COSTANTIN in dicotyledonous plants. If this author had extended his studies to the Monocotyledones, he would not have failed to notice the very strong development of the stereome in the stolons of numerous Gramineae and Cyperaceae, representing an epharmonic character of great importance.

The relatively ample leaves are held in a horizontal position, and the structure is bifacial. The cuticle is thin and smooth; the epidermis on the upper face is developed as large papillae all over the blade (fig. 7); while on the lower face the cells show the ordinary structure and are much smaller; the papillae are slightly thick walled. Stomata (fig. 10) abound on the lower face, raised somewhat above the adjoining epidermis, and the air chamber is very wide. Pointed, pluricellular hairs (fig. 6), papillose at apex, are frequent along the veins on the dorsal face. The chlorenchyma is composed of a single layer of short palisade cells covering an open pneumatic tissue of about three strata; large idioblasts containing aggregated crystals of calcium oxalate abound in the chlorenchyma close to the epidermis of both faces. A very characteristic structure is exhibited by the midrib; there are no papillae, the epidermis cells being small and thick walled on both faces. Some few strata of hypodermal collenchyma on the dorsal face, and a large strand on the ventral inclose a large, thin walled water storage tissue with some aggregated crystals. In the center of this tissue is a circular (in cross-sections) band of five or six collateral mestome strands, all turning the hadrome inward, and each strand is supported by an arch of stereome, not very thick

walled. The lateral veins of first order show the same structure, but contain only one mestome strand; the thinner veins of fourth or fifth order are surrounded by thin walled parenchyma sheaths, inside of which some few stereids are located on the leptome side. Tannin reservoirs were observed in the center of the stele in the midrib, as well as on the leptome side.

The leaves of the stolons are membranaceous, short, and tubular; the thin walled epidermis is perfectly glabrous, and there are no stomata and no water pores. There are about six layers of parenchyma in the middle of the leaves, containing a little starch, and some cells containing a brown gelatinous substance, especially in those close to the mestome strands, which are located in a single plane. They contain mostly leptome supported by a few stereids, but have no parenchyma sheaths.

The papillose ventral epidermis, papillose hairs, and midrib containing a stele are features characteristic of the aerial leaves of this species. Furthermore, the absence of the dark horseshoe-shaped spot upon the upper face of the leaves, so characteristic of the species of *Polygonum*, deserves attention. As a matter of fact this spot does appear upon the first developed leaves, which are unfolded before the locality becomes shady, but disappears soon.

SECTION PERSICARIA: *Polygonum pennsylvanicum* L., *P. lapathifolium* L., and *P. hydropiperoides* Michx.—The leaves show a very uniform structure in these species. They are unifacial with regard to the distribution of the stomata, bifacial with reference to the chlorenchyma, a typical palisade tissue of mostly two strata being well developed. Large idioblasts containing aggregated crystals of calcium oxalate were observed in the palisade tissue of *P. pennsylvanicum*, in the pneumatic tissue of *P. hydropiperoides*, and also in the ventral epidermis of the third species. Sessile glandular hairs with the head pluricellular were observed on both faces of the leaf in *P. lapathifolium*, but only on the dorsal in the other two species. Pluricellular papillose hairs like those of *P. virginianum*, but a little shorter, occur on the dorsal face of *P. lapathifolium*. The midrib contains a stele of six or seven separate collateral mestome strands in all three species, imbedded in a large strand of thin walled parenchyma, a water storage tissue. Tannin reservoirs and aggregated crys-

tals were observed in this tissue. There are a few strata of hypodermal collenchyma on both faces of the midrib, and an arch of rather thin walled stereome covers the leptome of the mestome strands in all the species.

In the stem of *P. hydropiperoides* the outer cell wall of the epidermis of the internodes forms numerous, very distinct, longitudinal crests, covered by the thin cuticle, a structure not observed in the other species. A hypodermal collenchyma of three or four separate strands occurs in *P. lapathifolium*, while in the other species this tissue forms a continuous sheath around the cortex proper, which is thin walled and destitute of chlorophyll. There is no distinct endodermis, but a closed stereomatic pericycle of one to three strata surrounding the stele. This contains a single band of many thick, collateral mestome strands alternating with some which are purely leptomatic. The pith is thin walled, starch-bearing, hollow in *P. lapathifolium*, solid in the others. Aggregated crystals of calcium oxalate were observed in the pith of all three species, but no tannin.

Characteristic of the nodes is the high development of the hypodermal collenchyma, being very thick walled and of many layers, about twelve in *P. pennsylvanicum*, and about eight in the other species. It represents the entire cortex in *P. pennsylvanicum* and *P. lapathifolium*, while in the third species the interior part of the cortex is thin walled, parenchymatic, and of three or four strata. No endodermis occurs in *P. lapathifolium*, but in the two others. It is thin walled and contains starch. While a very thin walled pericycle was observed in *P. pennsylvanicum*, this tissue is absent from the other species. The stele shows the same structure as the internodes, but the libriform is thin walled. The pith is large and contains starch in all the species; it is hollow in *P. lapathifolium*, solid in the others. Tannin was observed in the cortex and pith of *P. hydropiperoides*, none in the other species. Aggregated crystals were found in the pith of *P. pennsylvanicum* and *P. hydropiperoides;* sphaero-crystals in the pith of *P. lapathifolium*. These sphaero-crystals are quite abundant in the pith of the internodes and nodes, and they were also observed by SCHMIDT in some species of *Polygonum*. My material had been kept in alcohol for several months, and the crystals showed exactly the same structure as those of inulin, so common in the Com-

positae, and extended from one cell to another. They were soluble in dilute acids, partly so also in an aqueous solution of potassic hydrate, but water heated to 50° C. failed to dissolve them.

The peduncle of the inflorescence is glabrous in *P. hydropiperoides*, but the outer cell wall of the epidermis shows here also the sharp longitudinal crests. In the other two species the peduncle is very hairy, with the same types of hairs as observed in the leaves, long stalked glandular (fig. 9) or pointed papillose. The hypodermal collenchyma occurs as separate strands in *P. lapathifolium*, but as a continuous tissue in the two others; the inner three to seven layers of the cortex are thin walled in all the species, and in *P. hydropiperoides* this parenchyma is rich in aggregated crystals of calcium oxalate, besides many cells containing a brownish gelatinous substance. No endodermis was observed in *P. hydropiperoides*, but was in the two others, where several of the cells represented tannin reservoirs. A thin walled continuous pericycle surrounds the stele in all three species, and the structure of the stele is as previously described, including the very thin, purely leptomatic strands. Tannin reservoirs were found in the pith of all the species, besides aggregated crystals in *P. lapathifolium*.

SECTION AVICULARIA: *Polygonum aequale* Lindm. and *P. erectum* L.—*P. aequale* has recently been established by LINDMAN (11), in a paper on the so-called *P. aviculare* L., in which he divides this species into *P. aequale* and *P. heterophyllum*, in accordance with the material which this author has studied in the herbarium of CLIFFORT (Mus. Brit.), and in the herbarium of LINNAEUS, but labeled *P. aviculare* L. They are very distinct species according to the diagnoses and the excellent figures. Heterophylly is characteristic of the former, while the shape of the leaves is uniform in the latter; the perianth is almost cleft to the base in the former, while in the latter the lobes are much shorter (figs. 17, 18), and frequently even shorter than the basal, tubular part of the perianth. In the former the perianth is generally larger than the mature nut, but of the same length or a little shorter in *P. aequale*. The color and structure of the surface of the nut are more constant in these two species than the outline, being brownish or more or less blackish and dull with longitudinal striae in *P. heterophyllum*, and black or blackish, more or less shining, in-

distinctly striate or punctulate in *P. aequale*. Abnormal fruits are not seldom to be observed in the late fall, where the nuts are attenuated into a long point, and considerably longer than the perianth, besides being much narrower and of a pale greenish or yellowish color (fig. 19).

A third species, *P. calcatum*, has been described by the same author (12), but this is not to be considered as a variety of the old *P. aviculare*, but as a parallel species like *P. raji*, *P. bellardi*, etc. Characteristic of *P. calcatum* is the perianth, forming an oblong solid foot beneath the nut. The number of stamens is five, and the nut black, smooth, and shining. It is a native of Sweden, Lapland, Germany, southern Russia, western Asia, and the Himalayas; the other two species are evidently cosmopolitan. I have given this account of these plants since they are evidently widely distributed on this continent, and ought to be recorded as distinct species, as proposed by LINDMAN, instead of collective, so-called *P. aviculare* L. With reference to *P. aequale*, this is the species that occurs in the vicinity of Washington, D.C., along roads, especially in sandy or clayish soil in the open. *P. erectum* grows in gravelly soil in the open.

The leaves are unifacial in both species, not only with reference to the stomata, but also in regard to the chlorenchyma, representing a ventral and a dorsal palisade tissue. The leaves are held in a vertical position in *P. erectum*, partly also in the other species. No hairs were observed, and the epidermis is not papillose. In *P. erectum* the chlorenchyma consists of one layer of high palisade cells on both faces of the blade, inclosing a pneumatic tissue of roundish cells; in *P. aequale* there are two layers of high palisade cells on the ventral, and one to two strata, somewhat lower, on the dorsal face. Large idioblasts with aggregated crystals of calcium oxalate are frequent, especially in the dorsal palisade tissue of both species. Hypodermal strands of collenchyma were observed above and below the midrib, and also as arches covering the leptome of the mestome strands in *P. erectum*, while in the other species the collenchyma is confined to the dorsal face of the midrib. Four collateral mestome strands, arranged like a stele, and with the hadrome turned inward, constitute the midrib in *P. erectum*, while in *P. aequale* there is only one median mestome strand. The water storage tissue of the midrib is large in the former species and

very small in the latter. Tannin reservoirs form a closed sheath around the stele in *P. erectum*, but are absent from the other species. While no stereome occurs in *P. erectum*, a layer of this tissue covers the leptome of the midrib in *P. aequale*.

Some specimens of *P. erectum*, which grew in the shade (completely covered by a dense vegetation of very tall *Solidago*, *Erigeron*, and *Lactuca*) showed a somewhat modified structure, when compared with the plant from the open. The leaves were held in a horizontal position; the outer cell wall of the epidermis was slightly raised on both faces, although not papillose; the palisade tissue was less typical and less compact; and the collenchyma was less thick walled and not well represented. Finally, the water storage tissue was less developed, and did not contain any tannin reservoirs.

The internodes of the stem are glabrous in both species. While typical collenchyma is so well represented as hypodermal strands in *Polygonum* in general, in the section *Avicularia* a somewhat peculiar structure was observed. In *P. erectum* the hypodermal collenchyma is replaced by stereome, of which the cells, in cross-sections, show a very distinct interior ring bordering upon the lumen (fig. 15). When treated with chlor-zinc-iodine, however, the entire cell wall attains the same bright yellow color as the stereomatic pericycle and libriform in the same section. In *P. aequale*, on the other hand, the structure of this hypodermal tissue resembles more collenchyma in cross-sections, with the only exception of the cell wall showing a similar internal ring as in the preceding species. Viewed in longitudinal sections, however, the cells are long, and attenuated at both ends. Treated with chlor-zinc-iodine, the cell walls of the various strata, peripheral and internal, show a different reaction. In the three or four peripheral strata, the outer part of the cell wall becomes deep purple, while the internal ring remains bright yellow. In the interior three or four strata of this same tissue the complete cell wall becomes deep purple; in other words, the cells of the peripheral strata of this tissue are not purely collenchymatic, while those of the interior strata are so. The stereome covering the leptome of the collateral mestome strands, as well as the intra and interfascicular libriform, showed the typical reaction of true stereome in this species. This peculiar structure was observed and described as characteristic of *Poly-*

gonum "aviculare" by RÜTZOU (19), and the material examined, having been collected in Denmark, was evidently either *P. aequale* or *P. heterophyllum*.

When following the structure of this hypodermal tissue farther down toward the node, the reaction becomes more collenchymatic in all the cells, and the node itself shows this reaction in all the strata. The other part of the cortex in *P. erectum* is developed as a palisade tissue of one or two layers, surrounding some few strata of round cells rich in starch, and many of these contain the brownish gelatinous substance. In *P. aequale* the cortical parenchyma is of three or four strata of isodiametric cells, here and there interspersed with palisade cells, placed obliquely to the surface, and destitute of starch. There is a distinct endodermis in *P. aequale*. A stereomatic pericycle surrounds the stele in the manner of separate arches on the leptome side in both species. The mestome strands constitute a single band, alternating with some purely leptomatic in *P. erectum*, while in *P. aequale* the latter type, leptomatic, is absent. The pith is solid, rich in starch, and with many cells filled with the brown gelatinous substance in *P. erectum*, while neither this substance nor starch was observed in *P. aequale*.

In the specimens of *P. erectum* from a shady situation, the palisade tissue was less typically developed, and none of the cells of the cortex contained starch, or the brown gelatinous substance; on the other hand, aggregated crystals of calcium oxalate were abundant in the pith.

Before leaving this section, attention should be called to a paper by GREVILIUS (9), in which an interesting account is given of the stem structure of the formerly so-called *P. aviculare*. According to this author, stomata are more numerous and the palisade cells more typically developed in the forms collected in dry sunny situations than in those from shady or damp localities; the intercellular spaces in the palisade tissue are much wider in the forms from shady or damp localities than in those from the open. In specimens "cultivated" in the shade the cells of the cortex are either isodiametric or oblong, but parallel with the axis of the stem. Crystals of calcium oxalate abound in the forms from the open, but are absent from those grown in shady or damp situations. The stereomatic tissue shows the

same development in all the forms. The hadrome contained a larger number of vessels, and with the lumen much wider in the forms from the open, dry situations than in those from damp or shady places. The pith represents the greatest width in specimens from damp or shady situations. No mention was made of the occurrence of tannin.

SECTION CEPHALOPHILON: *Polygonum arifolium* L. and *P. sagittatum* L.—The leaves of both species are unifacial with regard to the distribution of the stomata, and these are raised high above the epidermis in *P. arifolium*, but level with the epidermis in the other species. Sessile glandular hairs with the head pluricellular (fig. 14) abound on both faces of the leaf in *P. arifolium*, besides large stellate hairs (fig. 14), and this type of hair is not mentioned by SOLEREDER as occurring in the Polygonaceae. In *P. sagittatum* glandular hairs like those of the former species are distributed over the dorsal face, together with some very rigid pluricellular, pointed, and curved hairs (fig. 8). The chlorenchyma shows the same structure in both species, namely, one layer of high palisades covering an open pneumatic tissue of three strata, and large idioblasts containing aggregated crystals of calcium oxalate are scattered throughout the chlorenchyma. The midrib contains hypodermal collenchyma on both faces, and a large water storage tissue surrounds a stele of five collateral mestome strands inclosing a broad strand of parenchyma like a pith. There are several tannin reservoirs in the water storage tissue, some located near the leptome, others in the center of the stele. An arch of stereome covers the leptome of all the mestome strands.

The internodes of the stem are glabrous in *P. arifolium*, but covered with curved, pluricellular, pointed hairs in the other species. Hypodermal collenchyma in two or three continuous strata was observed in both species, but no chlorophyll-bearing parenchyma. A thin walled endodermis was observed in the apical and basal internodes of *P. arifolium*, but not in the other species. A pericycle of thin walled stereome in a few layers surrounds a band of numerous, thick, collateral mestome strands in *P. arifolium*, while in the other species there are only four thick strands, the others being very thin, but nevertheless of the collateral type. Purely leptomatic strands are numerous in both species, and located in the same band as the collateral. The pith is solid in both species, rich in starch of large

grains. Tannin was observed in the endodermis and pith of *P. arifolium*, in the pith alone in the other species.

Characteristic of the node in *P. arifolium* is the highly developed collenchyma, which represents a broad zone of about fifteen strata, besides that the pericycle is purely parenchymatic, and thin walled. Furthermore, the pith contains numerous aggregated crystals of calcium oxalate, and some few tannin reservoirs; otherwise the structure agrees with that of the internode. In *P. sagittatum* the cortex consists of a single stratum of collenchyma, and of two or three layers of thin walled parenchyma with no deposits of starch. The pericycle is also parenchymatic in this species, and the stele shows the same structure as previously described. Tannin reservoirs were present in the pith.

In the species examined, *P. virginianum* and *P. hydropiperoides* being the only perennials, the roots are all of the nutritive type, and the structure is relatively very uniform. The primary root of *P. sagittatum* from low moist ground, and *P. pennsylvanicum* from dry open fields shows the same structure, pericambial cork, but no secondary cortical parenchyma, and the stele containing much thick walled conjunctive tissue. In *P. erectum* the primary root develops not only pericambial cork, but also secondary cortical parenchyma as a broad zone of about eight strata, and in which stereome appears in the form of small isolated strands, arranged in a single band. Many cells of the cortex contain the brown gelatinous substance, and the greater mass of the stele consists of thick walled libriform. In *P. aequale* the primary cortex persists and no secondary increase takes place outside the stele. The secondary roots of *P. hydropiperoides* have a broad primary cortex of about ten strata, traversed by wide lacunae. The secondary tissues are only represented by homogeneous, thin walled strata of cork, developed from the pericambium, and by the stele, in which thick walled conjunctive tissue is much in evidence. No tannin reservoirs were observed in any of these roots, and no aggregated crystals.

Discussion

When comparing the leaf structure of these species of *Polygonum*, it is noticed that *P. virginianum* is the only one in which the leaf structure is bifacial, with the stomata confined to the lower face, and

with the chlorenchyma differentiated into a ventral palisade and a dorsal pneumatic tissue. On the other hand, a unifacial structure as to the distribution of the stomata and the development of palisade cells in the ventral and the dorsal part of the blade is characteristic of *P. erectum* and *P. aequale;* in the other species the unifacial structure depends only upon the distribution of the stomata. The papillose epidermis is characteristic of *P. virginianum* alone, but in specimens of *P. erectum*, growing in a shady situation, the outer cell wall of epidermis was slightly raised, although without forming papillae. Moreover, the absence of glandular hairs in *P. virginianum* deserves attention, since this type of hairs is so well represented in the heliophilous species except *P. erectum* and *P. aequale*, in which the leaves and stems are perfectly glabrous. The structure of the palisade cells, being relatively very short in *P. virginianum*, is characteristic of this species, but does recur in the specimens of *P. erectum* from a shady situation. In all the other species the palisade cells show the typical structure, being high and compact. With regard to the collenchyma and the water storage tissue in the midrib, however, *P. virginianum* agrees with the other species. The midrib also represents a stele of several (four to six) collateral mestome strands in all the species except *P. aequale*, which has only one. Finally, tannin reservoirs were noticed in the midrib of all the species except in *P. erectum* from the shade, and in *P. aequale*.

With reference to the structure of the internodes, the relative development of the collenchyma and the thin walled parenchymatic cortex is about the same in the various species, except that a palisade tissue was observed in *P. erectum* and partly also in *P. aequale*. Also, the hypodermal collenchyma in the former species is replaced by stereome, and in the latter by a tissue which is not purely collenchymatic. An endodermis was observed in the internodes and nodes of *P. virginianum*, as well as in some of the other species; a stereomatic pericycle was observed in all the species examined, and mostly as a continuous sheath. The structure of the stele is also very uniform, purely leptomatic strands being interspersed with collateral, except in *P. aequale*, where all the strands are of the collateral type. A typical pith is developed in all the species, frequently with deposits of starch in large grains. Tannin reservoirs were especially abundant in the pith of the

internodes and nodes of *P. virginianum*, but only in the aerial; they occur also in the endodermis. In most of the other species tannin was noticed in the endodermis, the pericycle, and especially in the pith, but was absent from *P. erectum* in the shade, and also from *P. aequale*. Aggregated crystals of calcium oxalate were quite abundant in the cortex and pith of the aerial internodes of *P. virginianum*, and also in the pith of *P. erectum* from the shade; while they were absent from the typical specimens collected in the open. No crystals were observed in any part of the stem of *P. aequale*. The presence and distribution of crystals of calcium oxalate may not be constant, however, since GREVILIUS found these only in stems of *P. "aviculare,"* growing in the open and fully exposed to the sun; they appeared to be totally absent from specimens grown in the shade or in wet ground. In my material of *P. aequale*, collected in the open, no crystals were observed; on the other hand, they were quite abundant in *P. arifolium*, but entirely absent from *P. sagittatum*, although both species are inhabitants of bogs, wet places, etc.

According to SOLEREDER (20) only a small number of species of *Polygonum* are known from an anatomical point of view, but apparently the structure of the vegetative organs is very uniform. Among the characters recorded in the preceding pages, two appear to be new, namely, the stellate hairs in *P. arifolium* and the papillose epidermis in *P. virginianum*. Regarding the root structure, which is not mentioned in SOLEREDER'S work, nothing of importance was found except that the secondary cortex contains separate strands of typical stereome in *P. erectum*.

While the section *Tovara* is monotypic on this continent, a second species, *P. filiforme*, is credited to Japan. THUNBERG, however, did not describe the very important fruiting stage, and from the brief characterization of the section given by DAMMER it is not certain whether the Japanese species is distinct from the American, or merely representing a form of this. BENTHAM and HOOKER considered them closely allied. As a section *Tovara* is quite distinct from the others, notwithstanding the fact that the floral structure, the four-parted perianth, the number of stamens fluctuating between four and six, and the two styles recurs in the section *Tephis* Adans. This section, however, is closely related to *Avicularia*, hence the

habit is entirely distinct; moreover, the final structure of the styles is very different. Concerning the internal structure of the vegetative organs, *Tovara* possesses some truly epharmonic characters, expressed by the papillose leaf epidermis and the short, plump palisade cells, none of which occur in the heliophilous species examined. The peculiar hairs, the large idioblasts containing aggregated crystals of calcium oxalate, the tannin reservoirs, and the midrib of the leaf representing a stele may be regarded as simply fixed, since they recur in the heliophilous species of the genus. When we compare the structure of *P. virginianum* with that of the specimens of *P. erectum*, grown in the shade, the analogy points toward the possibility that the characters they have in common are epharmonic, and especially adapted to shady situations. With regard to the species from the open, the unifacial leaf structure with palisade cells on both faces, as represented by the section *Avicularia*, corresponds well with the foliage being held in an erect or at least ascending position, thus fully exposed to the light, and is evidently an epharmonic character. In the other species the horizontal position of the leaves is accompanied by a dorsiventral structure of the chlorenchyma, as is generally the case. Such structures as the stellate hairs in *P. arifolium*, however, the crested epidermis in *P. hydropiperoides*, and the stereomatic collenchyma in *Avicularia* cannot be accounted for, except as purely specific structures.

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EXPLANATION OF PLATES I, II

Polygonum virginianum

PLATE I

FIG. 1.—Subterrannean stem with stolons and one aerial shoot (*St*); natural size.

FIG. 2.—Diagram of flower: *R*, main axis.

FIG. 3.—Flower; enlarged.

FIG. 4.—Two perianth lobes with stamens; enlarged.

FIG. 5.—Fruiting stage; nut inclosed within persisting perianth; enlarged.

FIG. 6.—Hair from dorsal face of leaf; ×320.

FIG. 7.—Cross-section of leaf: *Ep*, epidermis; *P*, palisade tissue; ×320.

FIG. 8.—*P. sagittatum:* hair from leaf; ×240.

FIG. 9.—*P. pennsylvanicum:* hair from apical internode beneath inflorescence; ×360.

PLATE II

FIG. 10.—Dorsal epidermis of leaf with stoma; ×320.

FIG. 11.—Longitudinal section of pith of internode, showing tannin reservoir and cells with aggregated crystals of calcium oxalate; ×240.

FIG. 12.—Cross-section of pith of internode, showing two tannin reservoirs and two cells with crystals; ×240.

FIG. 13.—Cross-section of part of middle internode: *St*, steromatic pericycle; *L*, interfascicular cambium and libriform, and strand of pure eptome; *P*, pith; ×320.

FIG. 14.—*P. arifolium:* glandular and stellate hair from dorsal face of leaf; ×480 and 360.

FIG. 15.—*P. erectum:* cross-section of peripheral part of internode; *Ep*, epidermis; *C*, cortex; *St*, stereome; ×480.

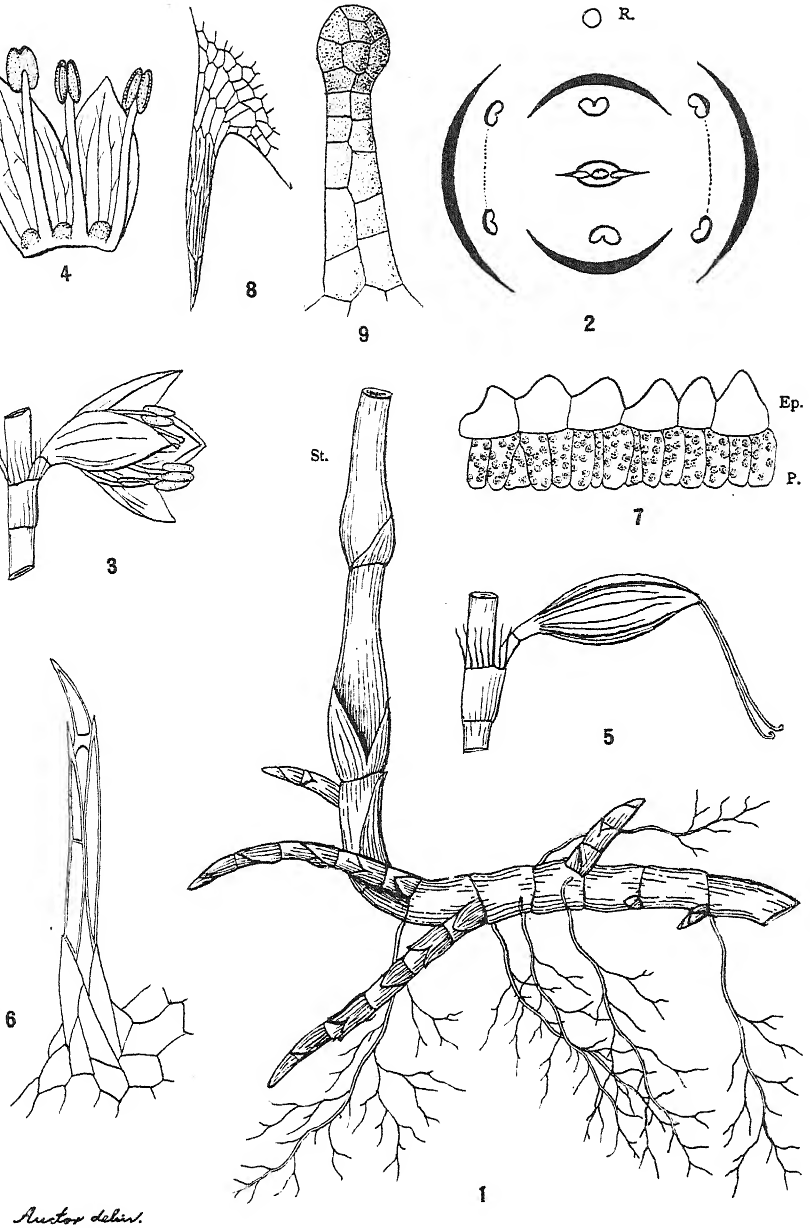
P. aequale

FIG. 16.—Leaf; twice natural size.

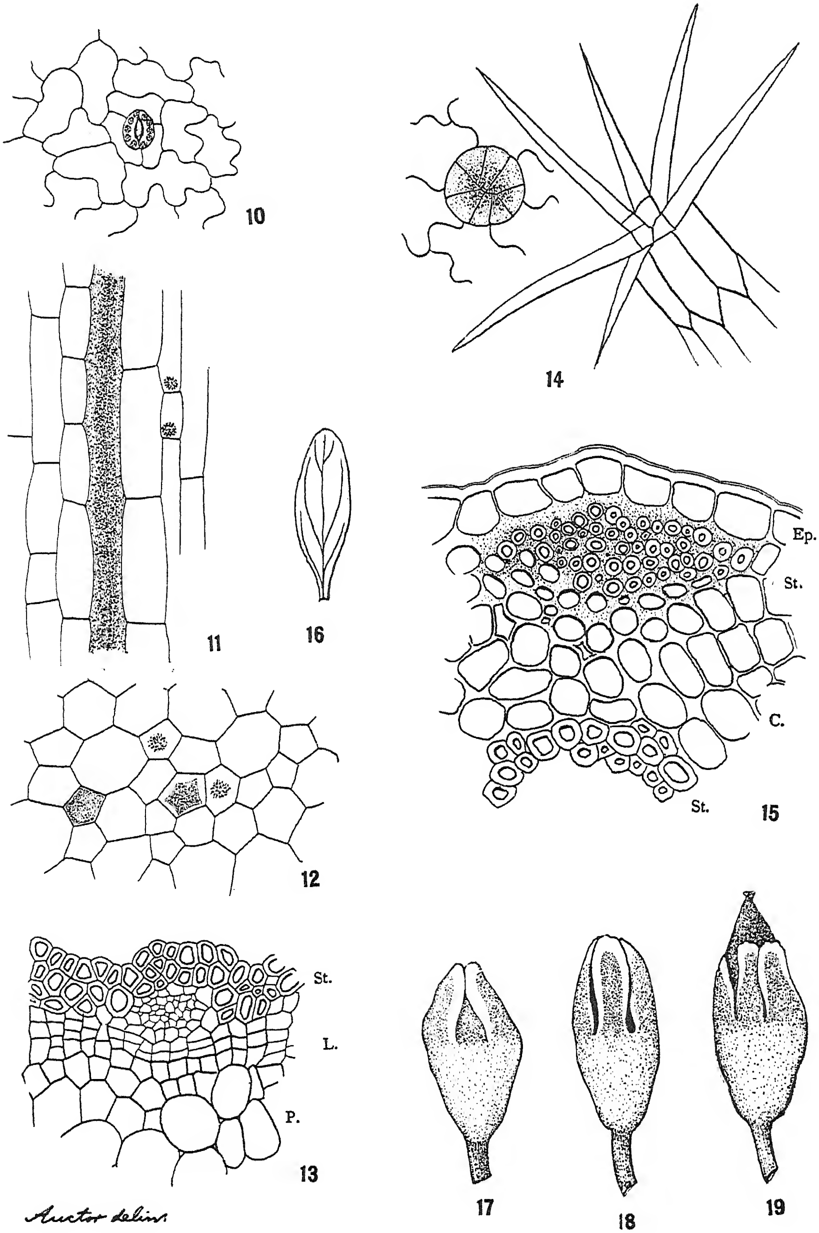
FIG. 17.—Flower bud; ×8.

FIG. 18.—Mature flower with nut hidden within persisting perianth; ×8.

FIG. 19.—Abnormal flower with fruit extending beyond perianth, collected late in fall; ×8.



HOLM on POLYGONUM



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HOLM on POLYGONUM

SEXUAL DIMORPHISM IN MUCORALES

I. INTRASPECIFIC REACTIONS

A. F. Blakeslee, J. L. Cartledge, D. S. Welch, and A. D. Bergner

Introduction

In a previous paper (7) experimental evidence for a strict sexual dimorphism in *Cunninghamella* has been given in some detail. The literature bearing on the question of sexuality in the Mucors is there cited, especially as it offers evidence in regard to sexual dimorphism in this group of fungi. In a still earlier publication (5) detailed tests are given for a single species of *Absidia*, as well as a provisional summary of tests with other forms. The methods employed in collecting the races studied, making the tests, and tabulating the reactions have already been described (6). The purpose of the present paper is to give in a series of tables the results of testing races within species other than those already recorded. In a second paper in this issue are given the results of testing together races belonging to different species.

Most of the tests recorded were made during the years 1919–1923. Unless otherwise noted, the cultures were run at laboratory temperature on our standard nutrient, no. 230, consisting of 2 per cent each of agar, dry malt, and dextrose, with 0.1 per cent peptone. Other nutrients used were no. 360, consisting of no. 230 plus 0.5 per cent dried horse dung decoction; no. 362, consisting of agar with addition of 2 per cent whey powder plus 1 per cent dextrose; no. 388, consisting of agar with 6 per cent malt and 1 per cent dextrose; no. 391, consisting of 3 per cent dextrose with 1 per cent dried horse dung decoction.

In the tables the letters A to D are used to indicate the different strengths of sexual activity measured by the relative number of zygospores produced in the given contrasts. Each race has been given a final numerical grade, made up of the average of its reactions with the testers of opposite sex (grade A having the numerical rank 4,

and grade D a rank of 1), and the races have been arranged in the tables according to their final grading. Since the various species differ in the abundance of zygospore production, the grades are comparable only within a given species. A race which has failed to show a sexual reaction with any of the testers has been classed provisionally as "neutral." It has been shown (2), however, that the races listed as neutral may be merely sexually weak races which have not been contrasted with sufficiently strong testers to elicit a sexual reaction. In cases in which the scarcity of zygospores or their position in the culture suggested the possibility of infection, the contrasts have been repeated. Contrasts between races with like signs are not represented in the tables, since no reactions have been found in such combinations.

The sources for the *Mucor* cultures indicated as T and H numbers in the various tables may be found in our earlier paper (7), except T 43 from a squirrel's nest in Cold Spring Harbor; T 71 from orange skins, Cold Spring Harbor; and H 3 from Brazil nuts from Huntington, New York. Where no T number is given, the origin of the culture may be found in the text. Several of the races studied were obtained from the Centralstelle für Pilzkulturen (1).

Experiments

ABSIDIA BLAKESLEEANA Lendner.—This species, recently described by LENDNER (11), has been discussed in an earlier paper (5) under the provisional designation "dark" *Absidia*, and a table has been given showing the strengths of reactions between the different races. Nineteen races were found to be (+), 18 (−), and 3 neutral. All the 40 races there listed were obtained from the same Brazil nuts which furnished the *Cunninghamella bertholletiae* previously reported (7), except race no. 638 which was supplied by Dr. THAXTER from a culture from Panama. Contrasts were run at oven temperature of about 30° C.

ABSIDIA CAERULEA Bainier.—Of the cultures listed in table I, races 911 and 912 were isolated from a culture producing zygospores on rabbit dung collected from Mt. Katahdin, Maine, in 1902; races 913 and 914 were supplied by the Centralstelle as mated strains under the name of *A. orchidis* and listed as coming from HAGEM; races 434–445 and 531–537 were isolated from ten samples of soil from

different localities in Cold Spring Harbor. All the 231 possible combinations were made with the twenty-two races. As shown in table I, four races were (+), thirteen were (−), and five (436, 437, 440, 441, and 443) not shown in the table were neutral. The cultures were run at about 26° C.

ABSIDIA CYLINDROSPORA Hagem.—The two races of this species were secured from the Centralstelle and were listed as coming from Hagem. The zygospores have been obtained on our nutrient 362 at a temperature of about 24° C.

ABSIDIA DUBIA Bainier.—This *Absidia*, which had been provisionally listed by us as "Cymose" *Absidia*, has been kindly determined by Professor LENDNER as probably *A. dubia*. The zygospores

TABLE I

ABSIDIA CAERULEA

GRADE	(+) RACES	(−) RACES												
		533	534	912	914	532	535	434	445	531	439	444	435	442
2.31......	913	B	C	B	B	B	C	B	C	C	C	B	D	D
2.23......	911	A	B	A	B	B	B	C	C	C	C	D	O	O
2.07......	536	B	B	C	B	C	B	C	B	C	C	C	O	O
1.54......	537	C	B	C	C	C	C	C	D	C	D	D	O	O
Grade..	......	3.00	2.75	2.75	2.75	2.50	2.50	2.25	2.00	2.00	1.75	1.75	0.25	0.25

are more or less spherical, and generally show either an equatorial ridge or more frequently a band which may have a width of about one-third of the zygospore. All our races were obtained from Brazil nut cultures, as indicated by the T and H source numbers (7). Difficulty was encountered at first in obtaining zygospore formation, and at the best the species does not seem to possess strong sexual activity. On account of the difficulty in distinguishing the races of this species from certain closely allied types, only races previously sexed by the imperfect hybridization reaction with other species were used. In consequence no neutrals appear in table II. Of the twenty-four races tested, four were (+) and twenty were (−). The cultures were run on nutrient 388 at about 30° C.

ABISIDIA GLAUCA Hagem.—Races 666 and 669 were isolated from the same sample of forest soil from Cold Spring Harbor; 917 and 918 were isolated by one of us from a zygosporic culture in the Harvard

Laboratory, and, as mated strains, have been kept under cultivation for about twenty years; 915 and 916 were supplied by the Centralstelle as mated strains of *A. glauca* and listed as coming from HAGEM; 919 was from the Centralstelle under the name *A. septata* and listed as coming from LENDNER; 920 was from the Centralstelle under the name *A. glauca* var. *paradoxica* and listed as coming from NAMYSLOWSKI. The cultures from the Centralstelle are here reported under the names they bore when received by one of us, and their origin is attributed to the authors later listed in the report of the

TABLE II

ABSIDIA DUBIA

Source				T43B	T101B	T50E	T55E	T76A	T71A	T50K	T50g	T54C	T114A	H3	$T74F_2$	T76B	T81A	T100	T100B	T75D	T113F	T115D	T115E
Grade				1.00	1.00	0.75	0.75	0.75	0.75	0.50	0.50	0.50	0.50	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
				(−) races																			
Source	Grade			597	599	376	382	383	598	375	378	379	798	206	361	384	388	390	391	596	797	800	801
T40F	1.05	(+) races	374	A	O	B	D	B	C	D	O	C	O	D	O	O	O	D	D	O	D	O	D
T113A	0.65		884	O	B	O	D	O	D	O	C	O	C	O	D	O	D	O	O	D	O	D	O
T50Ec	0.15		377	O	O	O	D	O	O	D	O	O	O	O	O	D	O	O	O	O	O	O	O
T99B	0.05		389	O	D	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O

Centralstelle (1) as having furnished the cultures in question. It is possible, of course, that contamination may have occurred or that errors may have crept into the records, so that the cultures originally supplied to the Centralstelle may not be, in every case, those that have been investigated by the present writers.

Table III shows the tests with *Absidia glauca*. All the forty-five possible contrast combinations were made with the total ten races. Of these four were (+), six were (−), and none failed to show a sexual reaction in at least two contrast combinations. The cultures were run at a temperature of about 26° C.

ABSIDIA RAMOSA Lendner.—Three cultures of *A. ramosa* (*typica*, var. *zurcheri*, and var. *rasti*) were obtained from the Centralstelle. The variety *zurcheri* is (−), and forms zygospores when con-

trasted with either of the two other races. The reaction between *typica* and var. *zurcheri* is stronger than that between var. *rasti* and the latter race. Cultures were run on nutrient 388 at about 33° C. Apparently the zygospores have not been reported previously. They are somewhat larger than those of *A. dubia*, being around $90 \times 60\mu$, laterally compressed, with usually at least three more or less distinct ridges, one equatorial and one or two on each side of the median ridge.

ABSIDIA REPENS Van Tieghem.—A pair of (+) and (−) races of this species has been in cultivation for about twenty years. The (+) came from a laboratory culture of Brazil nuts, the (−) from nuts

TABLE III

ABSIDIA GLAUCA

GRADE	(+) RACES	(−) RACES					
		666	669	918	916	920	919
2.33	915	B	B	C	C	C	C
2.33	667	B	A	B	C	C	O
2.17	668	B	B	B	C	C	O
1.83	917	C	D	C	C	C	C
GRADE	....	2.75	2.75	2.50	2.00	2.00	1.00

from the Island of Margarita, Venezuela. The zygospores are without outgrowths and resemble those of a small *Mucor*. Seven races from three soil cultures at Cold Spring Harbor were contrasted with these old (+) and (−) testers, and all were found to be (+). All the reactions were B except one which was C. The cultures were run on nutrient 362 at about 24° C.

"WHORLED" ABSIDIA.—This is a species common on Brazil nuts. LENDNER, to whom material was sent for identification, writes that it corresponds to *A. cylindrospora* Hagem. Since, however, the (+) and (−) races of the latter species, obtained from the Centralstelle, form zygospores with each other but fail entirely to form zygospores with the species under discussion, it seems best, until further tests can be made with a larger collection of races of *A. cylindrospora*, to call our species provisionally "whorled" *Absidia*. All the 528 possible combinations between the total 34 races were made.

Fourteen races were (+), eighteen were (−), and two (685 and 686, from Brazil nuts obtained from Knoxville, Tennessee) were neutral, and are omitted from table IV. The cultures were run at about 27° C.

BLAKESLEA TRISPORA Thaxter.—Two mated strains of this species were kindly sent us by GEORGE F. WEBER, who had isolated them in Gainesville, Florida, from poured agar plates upon which had been planted unsterilized sclerotia of another fungus, as reported

TABLE IV

"WHORLED" ABSIDIA

			Source	T96	T116	T96	T99	T113	T114	T73	T114	T114	T97	T99	T66	T98	T66	T98	T79	T96	T115
			Grade	3.14	2.64	2.57	2.57	2.50	2.50	2.36	2.36	2.29	2.21	2.21	2.07	2.07	2.00	1.93	1.71	1.21	0.79
				(−) races																	
Source	Grade			400	687	635	807	678	681	634	680	682	402	405	251	403	394	404	398	399	809
T68	2.89	(+) races	395	B	B	A	A	A	A	C	B	B	B	B	B	C	B	C	B	C	D
T68	2.72		397	B	A	B	A	A	A	B	B	B	C	C	B	B	C	C	C	D	D
T52	2.61		392	A	B	B	B	C	A	C	A	A	B	C	C	B	B	C	C	D	O
T52	2.61		393	B	A	B	A	A	B	B	C	B	C	C	B	C	C	C	B	C	O
T116	2.39		684	A	C	A	C	C	C	B	C	C	B	B	C	B	C	C	C	C	D
T111	2.28		808	A	B	C	C	C	C	B	B	C	B	B	C	B	C	C	D	D	D
T96	2.22		636	A	B	C	C	C	C	C	B	B	C	C	B	C	C	C	C	C	O
T68	2.17		396	C	B	B	B	B	C	C	C	C	C	C	C	C	C	C	C	C	D
T116	2.17		688	A	C	B	B	C	C	C	C	C	B	C	C	C	B	C	D	D	D
T111	2.06		676	B	C	C	C	C	C	B	C	C	C	C	C	C	C	C	C	C	D
T113	1.89		679	B	C	B	C	C	C	C	C	C	C	C	C	C	C	C	D	O	D
T97	1.83		401	C	B	C	B	B	B	C	C	C	D	C	C	D	D	C	O	O	C
T116	1.83		683	B	C	C	C	C	C	C	C	C	C	C	D	C	C	C	C	O	D
T112	0.78		677	C	D	O	O	D	D	C	D	O	D	C	O	O	O	D	D	D	O

by him at the Kansas City meeting of the A.A.A.S. Mr. WEBER writes that he has again isolated the two strains from plated portions of cucumber leaves. Zygospores are produced in abundance on various media at laboratory temperatures.

CHAETOCLADIUM BREFELDII Van Tieghem and Le Monnier (*C. jonesii* Brefeld).—Twelve races of *Chaetocladium brefeldii* were obtained from nine different cultures of horse dung at Cold Spring Harbor. One culture was sent us by Dr. WESTON from the Harvard laboratory. We have not obtained a true imperfect hybridization between the sexual races of *Chaetocladium* and known (+) and (−) races of other mucors, although such a reaction has been reported

by BURGEFF (9). As yet, therefore, we are unable to know which sex of this parasite is (+) and which (−). In consequence, the sex of the races is not given in table V and in the summary table XIII. Possibly biochemical tests, which have not yet been made with this species, may enable us ultimately to assign the terms (+) and (−) to the races which we have under cultivation (cf. under *Parasitella*).

All possible combinations were made between the thirteen races. Five proved to be of one sex and eight of the other. The strength of reactions is shown in table V. The host used was a *Mucor* of the "*mucedo*" type, which was grown at a temperature of 15°−20°C. *Chaetocladium* is able to grow saprophytically on the usual media at laboratory temperatures, however, and to produce zygospores without a host.

TABLE V

CHAETOCLADIUM BREFELDII

Grade	Grade	3.00	2.60	2.20	2.20	2.00	1.60	1.20	1.00
		951	952	947	954	959	958	949	956
3.25	957	A	A	A	A	B	C	C	B
2.63	948	B	A	C	A	B	A	O	D
1.63	950	B	D	B	O	D	D	B	D
1.63	953	B	C	C	B	B	O	O	O
0.75	955	C	C	O	O	O	D	D	O

CHOANEPHORA, species A.—In 1906 Professor F. L. STEVENS of Raleigh, North Carolina, kindly gave us a tube of this undetermined species of *Choanephora* which was producing zygospores abundantly. The (+) and (−) races were isolated and kept under cultivation for several years, but eventually died out. The zygospores are similar to those of *C. cucurbitarum* but larger, measuring from 57×50 to $103\times100\mu$, with an average of about $74\times70\mu$. In an earlier publication (4) may be found a photograph of a culture showing the two races of this species producing a line of zygospores where they have grown in contact, and imperfect hybridization reactions with another species.

CHOANEPHORA CUCURBITARUM (B. and Rav.) Thaxter.—This *Choanephora* is commonly found growing on withered flowers or cucurbits. The races used in the tests recorded in table VI were ob-

tained from cultures of withered flowers of squash and pumpkin collected from seven different gardens within a radius of about eight miles from the laboratory at Cold Spring Harbor. In all, ten races were used as testers, including five (+) and five (−) races, and a total of 275 contrast combinations were made with the thirty-three races studied. Of these five were (+), twenty-eight were (−), and none failed to show reactions in some of the combinations. Since all of the (+) races were used as testers, it has not been necessary to

TABLE VI

CHOANEPHORA CUCURBITARUM

Grade	(−) races	(+) testers 821	835	849	820	836	Grade	(−) races	(+) testers 821	835	849	820	836
3.60.....	834	B	A	B	A	A	3.20...	844	A	B	B	B	B
3.60.....	843	A	A	B	B	A	3.20...	846	A	B	B	B	B
3.40.....	816	A	A	B	B	B	3.00...	823	A	C	B	B	B
3.40.....	832	A	B	A	A	C	3.00...	825	B	B	B	B	B
3.20.....	814	B	A	B	B	B	3.00...	837	B	B	B	B	B
3.20.....	819	B	A	B	B	B	3.00...	839	B	B	A	C	B
3.20.....	824	C	A	A	B	B	3.00...	840	B	B	B	B	B
3.20.....	826	A	B	B	B	B	3.00...	841	B	A	B	B	C
3.20.....	828	B	B	B	B	A	3.00...	845	A	B	B	B	C
3.20.....	829	B	A	B	B	B	3.00...	847	B	B	B	A	C
3.20.....	830	B	C	A	A	B	2.80...	818	C	B	B	B	B
3.20.....	831	B	B	A	B	B	2.80...	827	A	C	B	C	B
3.20.....	838	B	B	B	A	B	2.80...	833	B	B	B	B	C
3.20.....	842	A	A	B	C	B	2.60...	817	C	C	B	B	B
							Grade.	......	3.25	3.18	3.18	3.07	2.92

group together the (−) testers (832, 823, 837, 839, and 818). An inspection of the table shows that very little difference exists between the sexual activities of the several races. It is quite possible that the slight differences in the records may be due to small variations in the environmental conditions in the different dishes, or to inconsistencies in the assigning of grades. The cultures were run on nutrient 362 at a temperature around 27° C.

CIRCINELLA SPINOSA Van Tieghem and Le Monnier.—The reactions with this species are shown in table VII. Of the races for which no T and H numbers are given, 567 and 568 came from laboratory cultures at Harvard University; 569 and 570 came from sheep dung at Cold Spring Harbor; 813 from Brazil nuts purchased at

Montreal, Canada; 905 was obtained from a squirrel nest; and 923 from horse dung at Cold Spring Harbor.

TABLE VII

CIRCINELLA SPINOSA

(−) TESTERS	(+) RACES													
	417	424	426	558	555	556	560	564	570	418	421	422	425	
413 416	C B	C C	C C	A O	D C	D C	B O	B O	C D	C O	C O	C O	C O	
Grade.........	2.50	2.00	2.00	2.00	1.50	1.50	1.50	1.50	1.50	1.00	1.00	1.00	1.00	
Source........	T38	T51	T53	T81	T79	T80	T96	T101	—	T38	T50	T50	T52	

(−) TESTERS	(+) RACES													
	427	430	431	432	553	563	568	554	559	569	420	791	792	
413 416	C O	C O	C O	C O	C O	C O	C O	D O	D O	B —	C —	Z C	Z D	
Grade.........	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	0.50					
Source........	T66	T74	T75	T75	T74	T101	—	T79	T81	—	T40	T117	T117	

(−) TESTERS	(+) RACES													
	780	784	785	786	787	788	789	790						
413 416	Z O	Z O	Z O	Z O	Z O	Z O	Z O	Z O						
Grade.........	.....	.....	.....	.....	.....	.....	.....	.....						
Source........	T111	T115	T115	T115	T115	T116	T116	T117						

(+) TESTERS	(−) RACES													
	416	413	419	429	433	415	428	557	562	414	781	794	782	783
426 417	C B	C C	C C	C C	D C	C O	C O	C O	O C	D O	Z B	Z B	Z C	Z O
Grade.........	2.50	2.00	2.00	2.00	1.50	1.00	1.00	1.00	1.00	0.50	.....	.....	.....	.....
Source........	T27	H2	T39	T66	T76	H2	T66	T80	T99	H2	T111	T118	T112	T113

Of the total fifty-five races, thirty-four were (+), fourteen were (−), and seven races (412 (H_2), 423 (T 51), 567, 793 (T 117), 813, 905, and 923) which do not appear in the table, were neutral. Races 569 and 420 were lost before the second test was made. Races 780–

792 and 794 were tested in the first series, but their zygosporic reactions (Z) were not graded. The cultures were run at a temperature of about 26° C. on nutrient 362. In the tests with 416 and 417, the nutrient was modified by the addition of 0.1 per cent lactic acid, which had little if any influence upon the growth of the cultures. It was found necessary to inoculate the races very close together (1–2 mm. apart), since zygospores fail to form at any considerable distance from the points of inoculation.

CIRCINELLA UMBELLATA Van Tieghem and Le Monnier.—A pair of races of this species has been kept under cultivation for about twenty years. The (+) race came on a culture of rat dung from China, the (−) from a culture of dog dung from Cambridge, Massachusetts. Zygospores have been obtained on nutrient 362 at a temperature of about 24° C.

CUNNINGHAMELLA.—In our earlier paper (7), a detailed report was given of sexual reactions within the species *C. bertholletiae* Stadel, *C. echinata* Thaxter, *C. elegans* Lendner, and a form provisionally listed as *Cunninghamella* A.

HELICOSTYLUM PIRIFORME Bainier.—Races 860 and 861 were found on cultures of Brazil nuts purchased in Oyster Bay, New York, and New York City respectively. Races 862–865 and 869 were obtained from dung cultures from Cold Spring Harbor. Races 867 and 868 were from laboratory cultures and have been kept running for a number of years. The zygospores resemble those of *Mucor mucedo*, but are smaller. Uncoiled outgrowths resembling mycelial filaments may arise from the suspensors and extend their growth to a considerable distance. All of the thirty-six possible contrast combinations were made with the total nine races. Of these six were (+), three (−), and none was neutral. All the contrasts between (+) and (−) races gave either B or C reactions. Cultures were run on nutrient 362 at temperatures between 23° and 26° C.

MUCOR GRISEO-CYANUS Hagem.—Of the races of this species tested, a (+) came from the Harvard laboratory, and another of the same sex was obtained from a decayed pumpkin; of the (−) races, two were obtained from rabbit dung and one from a soil isolation culture from Cold Spring Harbor, two from decayed raspberries from Amherst, Massachusetts, and one from the Centralstelle. Of

the total nine races tested, three were (+) and six were (−). Ten combinations were made, and all the contrasts between (+) and (−) races gave either B or C reactions. Cultures were run on nutrient 362 at about 25° C.

MUCOR HIEMALIS Wehmer.—One (+) and one (−) race of this species were obtained from the Centralstelle and listed as coming from HAGEM. Zygospores formed readily on ordinary media at laboratory temperatures. The (+) race has died, but the (−) is still in cultivation.

MUCOR DISPERSUS Lendner.—This species, provisionally designated as *Mucor* "D" in earlier publications (12, 13), has been kindly determined for us by Professor LENDNER. It was obtained in Cold Spring Harbor from fallen leaves which had been sown on sterilized bread. Only a single pair of (+) and (−) races have been studied.

MUCOR MUCEDO Linnaeus.—The term *Mucor mucedo* has been applied probably to a number of distinct species among the unbranched forms with large sporangia. The type we have studied is of common occurrence on horse dung, from which source most of our races have been obtained. Our first (+) and (−) races were isolated in 1903 from a culture which was producing zygospores. By frequent sporangial transfers these (+) and (−) races were kept under cultivation in separate test-tubes for sixteen years. In 1918 the (−) race gradually became weakened in growth, and in 1919 it was impossible to obtain a transfer from the last culture which had reached the 213th sporangial generation. The (+) race died out in 1920, after it had reached the 218th non-sexual generation. It had not shown so much decline in vigor as the (−) race, and its dying out may have been assisted by an infection of mites. This (+) race had always been of greater vigor than its (−) mate, as judged by the fact that high temperatures had less marked effect upon its sexual activity. It has earlier been shown (2, 3) that cultivation of this species at temperatures such as are frequent in summer lessens the sexual activity, which may be regained when the races are grown at a low temperature for a few sporangial generations. For this reason, contrasts between the various races tested were made in a cold cellar. The cultures were run on nutrients 391, 360, and on sterilized horse dung.

The thirty-nine races tested represent thirteen gross cultures obtained from nine different localities. As shown in the summary table XII, 86 combinations were made; fourteen races were (+), eleven were (−), and fourteen were neutral. Because of the relatively few combinations made, no detailed table of reactions is given for this species. In addition to the races obtained from nature, we have included in the summary table XII the races of this species secured from zygospore germinations (3). The germ tube and spores in the germ sporangium from a zygospore of *M. mucedo* have been shown to be all of the same sex, either (+) or (−). The growths from 53 zygospores were sexed by contrasts between mycelia obtained from germ tubes or from streaks of the spores in their germ sporangia and the test (+) and (−) races. Of these, twenty-two were (+) and thirty-one were (−). In addition, the spores from germ sporangia from four other zygospores (one + and three −) were plated out and the resulting mycelia sexed. A total of 401 races from these monosporic cultures were (−) and twenty-four (+), but these figures obviously give no indication of the proportion of (+) and (−) germinations. The growths from thirty-four spores from the (+) sporangia were temporarily neutral and are included in the neutral column. By an error in making up the summary table in an earlier paper (5), the (−) and neutral races were unfortunately transposed.

In making tests of growths from zygospore germinations, two or more cultures were grown in a petri dish between the old (+) and (−) testers. In consequence each race functioned as a tester against one or more other races, in addition to the original (+) and (−) strains.

MUCOR N.—This undescribed species, which probably represents a new genus, was discovered by THAXTER on a culture of Brazil nuts in the Harvard laboratory. Its (+) and (−) races have been kept under cultivation for over twenty years. Zygospores have been obtained on nutrient 362 at about 27° C., as well as on other nutrients at laboratory temperature.

MUCOR III.—Mated races of this undetermined species of the racemose type came from a rat dung culture in the Harvard laboratory. The original (+) and (−) races have been kept under cultivation for over twenty years. In addition one (−) race was secured

from horse dung in Cold Spring Harbor and a (+) race each from Brazil nuts purchased in Hickory, North Carolina, and from a soil culture from Cold Spring Harbor. Four combinations were made. Neutrals in this species were not identified for the reason given under *Mucor* IV. Cultures were run on nutrient 362 at about 25° C.

MUCOR IV.—Of *Mucor* IV, an undetermined species of the racemose type, races 921 and 922 originally came from Cold Spring Harbor in 1902, and the (+) and (−) strains are still in cultivation. In

TABLE VIII

MUCOR IV

Grade	(+) testers	(−) races												
		577	878	922	930	857	871	874	875	881	41	880	876	879
2.54....	921	B	C	B	B	C	C	B	A	C	B	B	C	D
1.23....	855	C	B	C	C	C	C	D	O	C	O	O	O	O
Grade..	....	2.50	2.50	2.50	2.50	2.00	2.00	2.00	2.00	2.00	1.50	1.50	1.00	0.50

Grade	(−) testers	(+) races					
		921	873	855	872	877	882
2.33....	922	B	C	C	B	B	D
1.00....	857	C	C	C	O	O	O
Grade..	....	2.50	2.00	2.00	1.50	1.50	0.50

addition, 577, 855, 872–875, 878–882 were from soil collections from the vicinity of Cold Spring Harbor; 857 was from horse dung, 871 from undetermined dung from woods, 876 from sheep dung, and 877 from mouse dung, all from the neighborhood of Cold Spring Harbor. No. 41 was isolated from an infection of corn meal in Storrs, Connecticut. As shown in table VIII, thirteen (−) and six (+) races were contrasted with two (+) and two (−) testers each. On account of the difficulty of being sure of the identification of species in the racemose group, only those races which showed zygospores with at least one of the testers were used in this table; in consequence, no neutral races are listed. Cultures were run on nutrient 362 at a temperature of about 27° C.

MUCOR V.—*Mucor* V is a form found in 1904 on a culture of horse dung from North Carolina, and its (+) and (−) races have been kept in cultivation ever since. It is perhaps not specifically distinct from *M. hiemalis*, since it has formed zygospores, although in no great abundance, with the strains of this name obtained from the Centralstelle. It differs, however, rather markedly from the Centralstelle material in color. Originally its sexual vigor was extremely high and was therefore used as a tester in imperfect reactions with other species. At the present time it has considerably weakened in sexual activity. Two (+) races were obtained from a culture of Brazil nuts purchased in New York City, and a (−) race from the inside of a stump at Cold Spring Harbor. In all, ten combinations were made with the five races, of which three were (+) and two (−). The contrasts gave C and D reactions. No neutrals are recorded for the reason given under *Mucor* IV. Cultures were run at laboratory temperatures.

MUCOR VI.—The (+) and (−) races of this undetermined *Mucor* were isolated in 1904 from a horse dung culture in the Harvard laboratory. It formed zygospores on the ordinary media, but both races have died out.

MUCOR VII.—The (+) and (−) races of this undetermined species were isolated from a culture of guinea pig dung. Three other races came from a culture on deer dung from Storrs, Connecticut, and one from a soil culture at Cold Spring Harbor. Five combinations were made with the six races, of which one was (+) and five (−). The contrasts give three C and two D reactions. No neutrals were recorded for the reason given under *Mucor* IV. The cultures were run at about 25° C.

MUCOR VIII.—The (+) and (−) races of this undetermined species were isolated from zygosporic material found by THAXTER on cultivated plants of *Hesperis* in Cambridge, Massachusetts. Zygospores have been produced on nutrient 362 at a temperature around 27° C. The (−) race has died out within the last few years.

PARASITELLA SIMPLEX Bainier.—The sexual reactions of this parasitic *Mucor* have been discussed in an earlier paper (14). Race P I was obtained from heron dung in Cold Spring Harbor, P II from horse dung in Cold Spring Harbor, P III from horse dung from

Woodbury, Long Island, and P IV from horse dung from Long Beach. It has not been possible as yet to obtain a true imperfect hybridization between the sexual races of *Parasitella* and known (+) and (−) races of other mucors. From their biochemical behavior (14), however, we may provisionally assign the term (+) to race P IV and (−) to the other three races. The six possible combinations were made between the four races, of which one is listed as (+) and three as (−). The contrasts gave 1 A, 1 B, and 1 C reaction. The contrast series was run on *Mucor rouxii* as host, which was grown on nutrient 391 at laboratory temperature. Zygospore formation was found to be equally abundant when the cultures of *Parasitella* were grown saprophytically (14).

Phycomyces blakesleeanus Burgeff.—This is the common laboratory *Phycomyces*, a not infrequent growth on dung and universally cultivated under the name of *P. nitens*. Burgeff (10), however, has recently shown that the original *P. nitens* is a distinct species growing spontaneously on fatty material.

Our first (+) and (−) races were obtained in 1903 from rabbit dung from Cold Spring Harbor and North Carolina respectively. The (+) race is still in cultivation, having reached the 270th sporangial generation. The (−) race died out in 1923, however, when it had reached the 259th generation. Thirteen races were obtained from dung, chiefly of rabbit and horse in the neighborhood of Cold Spring Harbor. All the possible 105 combinations were made with the races, of which eleven were (+), one was (−), and three were neutral. The cultures were run on nutrient 360 at laboratory temperature.

In addition to these races obtained from nature, we have included, in the summary table XII, the races of this species obtained from isolation cultures from germ sporangia (3). They were tested in the same manner as were the races from zygospore germinations of *Mucor mucedo*. The segregation of sex in germ sporangia of *Phycomyces* is frequently incomplete, and spores are formed which give rise temporarily to bisexual or homothallic mycelia. These homothallic mycelia are not constant races, since they ultimately produce pure (+) and (−) spores in their sporangia. These temporarily bisexual mycelia (3) are characterized by the production of curiously contorted

yellowish outgrowths, and are properly considered by BURGEFF (8), from his grafting experiments, to be mixomycelia, or mycelia containing a mixture of (+) and (−) protoplasm. Such homothallic cultures, for convenience, are listed in table XII under the neutral column without meaning that such cultures are devoid of sex. No true neutrals have been found from this series of zygospore germinations, although a certain tendency toward temporary neutrality has been observed. A considerable increase in the number of races listed would occur if we had included streak cultures from germ sporangia and mycelia directly from germ tubes, as well as unpublished data on zygospore germinations in 1913.

RHIZOPUS NIGRICANS Ehrenberg.—The source of the races of *Rhizopus* which have been tested in the present study is given in table IX or in table X, where reference is made by T number to our previous paper (7).

Table IX shows tests with *Rhizopus nigricans*. Twenty races were used as testers, including ten (+) and ten (−) races, and in all 1574 contrast combinations were made with the total 97 races. Of these forty were (+), seventeen were (−), and forty failed to show reactions and were provisionally classified as neutral. This neutrality may have been due in part to the fact that species other than *R. nigricans* were probably included among the races tested (cf. table X).

The zygospores of *Rhizopus* have twice been brought to germination by one of us, once in 1906 and again 1913. On the first occasion the germ tubes produced small sporangia, the spores from which gave rise to weak mycelia which ultimately died. On the second occasion only germ tubes were formed which could be induced to produce neither sporangia nor mycelia. Races of *Rhizopus* have been collected and tested for sexual activity since 1904, and in consequence there is a considerable number of races recorded in the final summary in table XI which were contrasted with our test (+) and (−) races, but which were not used in the special study summarized in table IX.

SYNCEPHALASTRUM RACEMOSUM Cohn.—*Syncephalastrum* is a common infection on Brazil nuts along with *Cunninghamella*. Of the cultures for which a source number is not given, 658 was obtained from a gross culture of sheep dung from Cold Spring Harbor; 659

was from the Centralstelle and was listed as coming from ATKINSON; 660 to 663 were furnished by THAXTER and were derived from Florida, Porto Rico, South Carolina, and California respectively; 664 and 665 originated from Florida and Porto Rico respectively and are the races which have been kept under cultivation for about

TABLE IX

RHIZOPUS NIGRICANS

SOURCE "T" NOS.	GRADE	(+) RACES	(−) TESTERS									
			309	15B	106B	900	675	615	25B	305	674	303
—	3.70	899	A	A	A	A	A	C	A	A	B	A
—	3.20	15A	C	A	A	B	B	D	B	A	A	A
111	3.00	670	B	A	A	B	A	A	A	C	C	O
—	2.90	106A	A	A	A	O	B	A	C	A	O	A
100	2.90	410	B	A	D	B	C	B	B	B	A	B
—	2.60	304	C	B	C	B	A	B	C	C	B	C
—	2.60	314	A	C	C	B	A	B	B	C	B	O
80	2.60	343	B	A	D	A	C	B	D	C	A	C
81	2.50	344	C	B	C	C	A	B	C	A	O	C
—	2.40	306	C	A	A	A	O	B	A	B	O	O
27	2.40	323	O	A	B	B	O	A	A	O	A	C
—	2.20	25A	A	B	B	D	B	B	B	O	C	O
118	2.20	806	B	B	A	B	A	B	C	O	O	O
—	2.00	308	B	D	B	B	B	B	C	O	C	O
—	1.90	177	C	D	A	A	A	O	C	C	O	O
—	1.90	302	O	A	A	B	O	O	A	O	O	A
53	1.80	331	B	C	A	C	C	B	C	O	O	O
—	1.70	175	B	D	O	B	B	C	B	O	C	O
—	1.70	178	C	D	B	A	B	B	O	D	O	O
—	1.60	43	C	D	B	B	B	C	D	D	O	O
—	1.60	170	B	D	B	B	O	B	B	O	O	O
—	1.50	173	C	C	B	B	O	B	C	O	O	O
—	1.50	176	B	D	C	B	O	B	B	O	O	O
27	1.40	325	A	D	C	C	A	O	O	O	D	O
—	1.30	174	C	D	B	C	B	O	D	D	O	O
73	1.30	338	C	D	A	O	B	B	O	O	O	O
—	1.00	313	C	D	C	C	B	O	O	O	O	O
54	0.60	332	O	B	C	D	O	O	O	O	O	O
101	0.60	411	C	A	O	O	O	O	O	O	O	O
—	0.60	601	C	C	O	D	O	O	D	O	O	O
—	0.50	317	C	C	D	O	O	O	O	O	O	O
—	0.40	315	D	D	O	C	O	O	O	O	O	O
13	0.40	322	C	D	O	O	O	D	O	O	O	O
48	0.40	329	D	D	O	C	O	O	O	O	O	O
50	0.30	330	C	D	O	O	O	O	O	O	O	O
—	0.30	600	C	D	O	O	O	O	O	O	O	O
114	0.30	673	O	O	B	O	O	O	O	O	O	O
—	0.20	316	D	D	O	O	O	O	O	O	O	O
39	0.20	327	D	D	O	O	O	O	O	O	O	O
27	0.10	324	O	O	O	D	O	O	O	O	O	O
Grade			2.12	2.07	2.07	2.02	1.70	1.62	1.52	0.87	0.85	0.70

TABLE IX—*Continued*

Source "T" nos.	Grade	(−) races	(+) testers									
			106A	899	15A	670	304	343	25A	806	302	601
—	3.50	15B	A	A	A	A	B	A	B	B	A	C
—	2.90	106B	A	A	B	A	C	D	B	A	A	O
118..........	2.90	805	B	C	B	A	B	A	B	B	C	C
—	2.70	309	A	A	C	B	C	B	A	B	O	C
118..........	2.70	675	B	A	B	A	A	C	B	A	O	O
—	2.60	25B	C	A	B	A	C	D	B	C	A	D
—	2.60	900	O	A	A	B	B	A	D	B	B	D
—	2.40	102	A	C	B	O	B	C	B	B	C	C
—	2.30	615	A	C	D	A	B	B	B	B	O	O
76..........	2.20	337	A	B	A	O	C	D	A	C	O	C
—	2.10	307	A	B	A	C	C	A	O	C	O	O
—	2.00	303	A	A	A	C	C	C	O	O	O	O
—	2.00	305	A	A	A	O	C	C	O	O	A	O
76..........	1.90	341	B	A	A	B	B	C	O	O	O	O
115..........	1.80	674	O	B	A	C	B	A	C	O	O	O
—	1.60	616	C	O	O	A	C	D	B	C	O	C
—	1.60	103	C	O	O	A	C	B	C	O	O	B
Grade...	....	....	3.00	3.00	2.94	2.76	2.53	2.53	2.18	2.00	1.35	1.00

Source "T" nos.	Neutral races	Source "T" nos.	Neutral races	Source "T" nos.	Neutral races	Source "T" nos.	Neutral races
—	73	12	321	96	406	—	608
—	74	38	326	97	407	—	609
—	89	40	328	98	408	—	610
—	101	55	333	99	409	—	611
—	310	60	334	—	602	86	612
—	311	66	335	—	603	96	613
—	312	67	336	—	604	—	614
—	318	74	339	—	605	—	617
—	319	75	340	—	606	112	671
12	320	79	342	—	607	113	672

twenty years as the (+) and (−) representatives of *Syncephalastrum*. The zygospores are small, resembling those in *Mucor*.

Table XI shows the tests with *Syncephalastrum*. Eighteen races were used as testers, including eight (+), eight (−), and two neutral races, and in all 1269 contrast combinations were made with the total eighty races. Of these, thirty-seven were (+), thirty-nine were (−), and four were neutral. The neutrals 281 (T13) and 288 (T48), although contrasted with all the other races, are not listed with the other testers in the table. The zero reactions are also omitted between the (+) and (−) testers and the four neutrals 281, 288, 689

(T111), and 692 (T112). It will be observed that the (+) race 280 formed zygospores in only one of the contrast combinations. In consequence, if the (−) race 195 had not been used as a tester, race 280 would have been listed as a neutral. If the four races provisionally classed as neutral had been contrasted with other stronger testers, it is possible that they would all have given a (+) or a (−) reaction. Cultures were run at a temperature of about 27° C.

TABLE X

RHIZOPUS NIGRICANS

LIST OF RACES SHOWN IN TABLE IX FOR WHICH A SOURCE NUMBER IS NOT THERE GIVEN

RACE NO.	SUBSTRATUM	LOCALITY REPRESENTED	RACE NO.	SUBSTRATUM	LOCALITY REPRESENTED
15A & B	Bread	Durham, N.C.	312....	Strawberries	Georgia
25A & B	....	Amherst, Mass.	313....	Black raspberries	Connecticut
43.....	Boletus	Woods Hole, Mass.	314....	Black raspberries	Connecticut
73.....	Soil	New Brunswick, N.J.	315....	Cherries	Washington, D.C.
74.....	Soil	New Brunswick, N.J.	316....	Dewberry	Boston, Mass.
89.....	Soil	New Brunswick, N.J.	317....	....	Arkansas
101.....	....	Panama	318....	....	Arkansas
102.....	....	Panama	319....	....	Arkansas
103.....	....	Panama	600....	Almond nuts	Cambridge, Mass.
106A & B	Bread	Philadelphia, Pa.	601....	Lab. culture	Cambridge, Mass.
170.....	Dung and leaves	Southern China	602....	Bread	Toronto, Canada
173.....	Brazil nuts	Huntington, N.Y.	603....	Pistachio nuts	Catania, Italy
174.....	Almond nuts	Huntington, N.Y.	604....	Rhiz. oryzae	Centralstelle
175.....	Brazil nuts	Huntington, N.Y.	605....	Rhiz. oryzae	Centralstelle
176.....	Eng. walnuts	Huntington, N.Y.	606....	Rhiz. arrhizus	Centralstelle
177.....	Brazil nuts	Huntington, N.Y.	607....	M. norvegicus	Centralstelle
178.....	Brazil nuts	Huntington, N.Y.	608....	R. delamar	Centralstelle
302.....	Sweet potatoes	Huntington, N.Y.	609....	R. tritici	Centralstelle
303.....	Sweet potatoes	Huntington, N.Y.	610....	R. chinensis	Centralstelle
304B....	Sweet potatoes	Huntington, N.Y.	611....	R. nodosus	Centralstelle
305B....	Sweet potatoes	Huntington, N.Y.	614....	Brazil nuts	Amsterdam, N.Y.
306C....	Sweet potatoes	Oyster Bay, N.Y.	615....	Papaw	Cuba
307C ...	Sweet potatoes	Oyster Bay, N.Y.	616....	Artocarpus	Cuba
308.....	Strawberry	Washington, D.C.	617....	Anona	Cuba
309.....	Strawberry	Washington, D.C.	899....	Vegetables	Cambridge, Mass.
310.....	Strawberry	Washington, D.C.	900....	Vegetables	Cambridge, Mass.
311.....	Peaches	Boston, Mass.			

Summary and discussion

In table XII is given a summary of the heterothallic species tested for intraspecific sexual reactions. The classification is based almost exclusively upon the recorded tests made for the purpose of discovering the possible occurrence of sex intergrades. A considerable number of individual tests, made for other purposes during the last twenty years and more, have not been included in the table. Their inclusion would have greatly increased the number of combinations listed. Thirteen records, however, on eighteen races of the species previously classified, are listed separately. These for the most part are contrasts between races which had died out when the special con-

trast series were started and could not conveniently be included with the latter. Records are available for over 500 contrasts between 203 races of various species not otherwise listed. These were obtained in attempting to match up those races in our collection which were similar in appearance. Of the more than 500 combinations thus

TABLE XI

SYNCEPHALASTRUM RACEMOSUM

Source	Grade	(−) races	(+) testers							
			651	648	194	664	659	663	661	660
T81	3.00	653	A	C	C	B	A	A	C	B
T40	2.87	287	B	B	C	B	B	B	A	C
T96	2.87	655	A	A	C	B	B	B	C	C
T50	2.75	291	A	A	B	C	B	C	C	C
T74	2.75	301	A	A	B	B	C	C	C	C
—	2.75	658	A	C	C	B	B	A	O	A
T116	2.75	701	B	B	C	B	B	A	C	C
T115	2.75	707	B	C	C	A	B	B	B	C
T80	2.62	650	A	A	B	B	C	C	D	C
H2	2.50	195	A	A	B	C	C	D	C	C
T50	2.50	290	A	B	B	C	B	C	D	C
T74	2.50	299	B	C	C	B	A	C	C	C
H2	2.25	199	A	A	B	B	D	C	D	O
T51	2.25	293	A	B	C	C	C	C	D	C
T52	2.25	294	A	B	C	C	B	C	O	C
T81	2.25	652	A	A	B	C	C	D	D	D
T81	2.25	654	A	B	D	C	C	C	C	C
T118	2.25	708	A	B	C	B	D	C	C	D
H2	2.12	198	B	B	B	B	C	C	O	D
T13	2.12	279	A	B	C	D	C	C	D	C
T66	2.12	298	A	B	B	C	C	D	C	O
T55	2.12	641	A	B	D	B	C	D	C	D
T80	2.12	649	A	A	A	D	D	D	D	D
T112	2.12	691	C	B	B	C	C	C	C	D
T117	2.12	704	B	B	B	C	C	C	C	O
—	2.00	665	B	B	C	C	C	C	D	D
T112	1.87	693	C	A	C	C	D	D	C	D
T116	1.87	700	B	B	C	D	C	C	C	O
T117	1.87	706	A	A	C	B	O	O	C	O
T96	1.75	656	B	B	C	B	C	O	O	D
T114	1.75	698	C	D	B	C	C	C	C	O
H2	1.62	197	A	A	B	C	O	O	O	O
T12	1.62	276	A	A	B	C	O	O	O	O
T27	1.62	284	B	B	C	C	D	D	O	D
—	1.62	662	C	B	B	D	D	D	C	O
H2	1.37	200	A	B	C	C	O	O	O	O
T75	1.25	643	B	A	C	D	O	O	O	O
T114	1.12	699	D	D	D	D	D	C	C	O
T117	1.00	705	C	B	O	D	O	O	C	O
Grade			3.38	3.17	2.31	2.23	1.82	1.67	1.41	1.15

TABLE XI—*Continued*

Source	Grade	(+) races	(−) testers							
			652	195	653	658	705	287	662	665
T13.......	3.25	283	A	A	B	A	B	C	B	B
T80.......	3.25	651	A	A	A	A	C	B	C	B
T98.......	3.12	657	A	A	B	B	B	B	B	C
T12.......	3.00	277	A	B	B	A	A	C	C	C
T80.......	3.00	648	A	A	C	C	B	B	B	B
T113......	3.00	697	B	A	B	C	A	C	A	C
T54.......	2.87	297	A	B	B	B	B	C	B	C
T76.......	2.87	645	B	B	B	A	A	C	C	D
T113......	2.87	696	A	B	B	C	B	C	B	B
T118......	2.87	710	A	B	B	C	B	B	B	C
H2........	2.75	196	A	B	B	C	A	B	D	C
T13.......	2.75	282	A	B	B	B	A	C	D	C
T117......	2.75	703	A	A	C	C	A	B	B	O
T53.......	2.62	640	A	B	D	C	A	C	B	C
T118......	2.62	709	A	A	B	D	B	C	C	C
T51.......	2.37	292	B	B	B	B	C	C	C	D
T52.......	2.37	639	C	C	C	A	B	D	C	B
T79.......	2.25	646	A	B	C	D	B	C	C	D
H2........	2.12	194	B	B	C	C	O	C	B	C
H27.......	2.12	659	C	C	A	B	O	B	D	C
—........	2.12	664	C	C	B	B	D	B	D	C
T39.......	2.00	286	B	B	C	B	B	D	O	D
T79.......	2.00	647	B	C	C	B	C	D	C	D
—........	2.00	663	D	D	A	A	O	B	D	C
T76.......	1.87	644	B	D	C	B	C	D	C	D
—........	1.75	661	D	C	C	O	C	A	C	D
T113......	1.75	694	C	C	B	D	C	C	O	C
—........	1.62	660	D	C	B	A	O	C	O	D
T50.......	1.50	289	A	C	C	C	D	O	D	O
T27.......	1.25	285	D	C	B	O	D	C	O	D
T74.......	1.25	300	B	D	C	B	O	O	O	D
T54.......	1.12	296	C	D	D	B	D	D	O	O
T55.......	0.87	642	C	C	O	D	C	O	O	O
T111......	0.87	690	C	C	D	O	D	D	O	O
T113......	0.62	695	D	C	O	O	D	D	O	O
T12.......	0.37	278	D	D	D	O	O	O	O	O
T13.......	0.12	280	O	D	O	O	O	O	O	O
Grade.............			2.81	2.54	2.37	2.24	2.00	1.84	1.57	1.43

made, probably the majority represent intraspecific combinations. Since, however, in many cases we could not be certain, without the formation of zygospores, that all of a given group belonged to a single species, some of the contrasts may represent interspecific combinations, and it has seemed best to omit from table XII the number of combinations made within these groups.

The races listed under zygospore germinations of *Mucor mucedo*

and *Phycomyces* are derived in a different manner from those discussed earlier. Since there is presumably segregation following

TABLE XII

SUMMARY OF INTRASPECIFIC REACTIONS

	Locations	Gross cultures	Races	Combinations possible	Testers used	Combinations made	(+)	Neutral	(−)
Absidia blakesleeana	13	26	40	780	40	780	19	3	18
caerulea	3	12	22	231	22	231	4	5	13
cylindrospora	....	....	....	1	2	1	1	0	1
dubia	10	18	24	276	8	154	4	0	20
glauca	5	5	10	45	10	45	4	0	6
ramosa	....	....	3	3	2	3	2	0	1
repens	3	5	9	36	2	15	8	0	1
sp. (whorled)	11	15	34	561	34	528	14	2	18
Blakeslea trispora	....	....	2	1	2	1	1	0	1
Chaetocladium brefeldii	1	10	13	78	13	78	?	0	?
Choanephora sp. A	1	1	2	1	2	1	1	0	1
Choanephora cucurbitarum	2	19	33	528	10	275	5	0	28
Circinella spinosa	13	28	55	1485	4	206	34	7	14
Circinella umbellata	2	2	2	1	2	1	1	0	1
Cunninghamella sp. A	9	18	53	1378	6	297	22	2	29
bertholletiae	18	36	89	3916	15	1215	12	8	69
echinulata	7	10	18	153	18	153	10	0	8
elegans	1	16	42	861	12	426	25	1	16
Helicostylum piriforme	4	9	9	36	9	36	6	0	3
Mucor griseo-cyanus	5	8	9	36	2	10	3	0	6
hiemalis	....	....	2	1	2	1	1	0	1
dispersus	2	2	2	1	2	1	1	0	1
mucedo	9	13	39	741	4	86	14	14	11
sp. N	1	1	2	1	2	1	1	0	1
sp. III	3	4	5	10	2	4	3	0	2
sp. IV	5	13	19	171	4	76	6	0	13
sp. V	3	3	5	10	2	5	3	0	2
sp. VI	1	1	2	1	2	1	1	0	1
sp. VII	3	3	6	15	2	5	1	0	5
sp. VIII	1	1	2	1	2	1	1	0	1
Parasitella simplex	3	4	4	6	4	6	1	0	3
Phycomyces blakesleeanus	....	....	15	105	15	105	11	3	1
Rhizopus nigricans	....	....	236	27,730	20	1574	89	85	62
Syncephalastrum racemosum	18	35	80	3160	18	1269	37	4	39
Other races of above species	....	....	18	......	....	13	7	2	9
Races of species not listed	....	....	203	......	....	....	40	99	64
Totals	....	....	1111	......	296	7604	393	235	470
ZYGOSPORE GERMINATIONS									
Mucor mucedo	....	....	512	......	514	1280	46	34	432
Physomyces blakesleeanus	....	....	392	......	394	980	258	16*	118
Totals	....	....	904	......	908	2260	304	50	550
Grand totals	....	....	2015	......	1204	9864	697	285	1020

* Mixomycelia, not true neutrals.

sexual reproduction, an opportunity would be given among these races for the occurrence of sex intergrades which might not be able to survive in nature, which is the source of our other cultures. All the species in table XII (except the parasitic forms) have been tested by the imperfect hybridization reaction which will be discussed in a succeeding paper in this journal. The parasitic forms, *Chaetocladium* and *Parasitella*, failed to give a true imperfect hybridization reaction with the races with which they were contrasted. On account of their reaction to biochemical tests, it has been possible to assign provisionally the terms (+) and (−) to the opposite sexes of *Parasitella*. *Chaetocladium*, however, has not yet been tested biochemically, and it has not yet been possible, therefore, to list its sexual races in the (+) and (−) columns in the table.

Various grades of sexual vigor are apparent in the different races tested within a single species. While in general there is a more or less orderly decrease in strength of reaction as one goes from contrasts between strong to those between weak races, still there is evidence from the tables for a certain amount of compatibility and incompatibility between certain races. As has been pointed out before, the races listed as neutral are probably, in many cases at least, (+) and (−) races, so weak sexually that none of the testers of the other sex yet contrasted with them has been able to call forth zygospore formation. Thus, in table IX, if the (−) tester 195 had not been used with the (+) race 280, the latter would have been listed as a neutral.

The investigations reported in the present paper were undertaken with the purpose of discovering whether among the heterothallic Mucorales sex intergrades could be found such as are frequently encountered in higher plants. Records are given of tests with over 2000 races included in thirty-four or more species included in twelve genera. If the tests not listed in table XII are included, between 10,000 and 20,000 intraspecific combinations have been made between heterothallic species, without evidence for sex intergrades. We may conclude, therefore, that so far as our tests have gone, the heterothallic mucors are sexually strictly dimorphic.

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SEXUAL DIMORPHISM IN MUCORALES

II. INTERSPECIFIC REACTIONS

A. F. BLAKESLEE AND J. L. CARTLEDGE

In the preceding paper in this series (5) were given the results of testing together races belonging to the same species. When two sexually active races of the same species are allowed to grow together under proper environmental conditions, zygospores ordinarily result. In distinction from the perfect reactions in these intraspecific contrasts, imperfect reactions may occur in interspecific contrasts, when the two sexually active races belong to different species. Interspecific reactions have been discussed in earlier publications (1, 2, 3) under the term "imperfect hybridization." The reaction may be indicated merely by the formation of small progametes at the point of contact between the (+) and (−) hyphae of the two species. Sometimes the gametes (gametangia) are delimited from one or both of the conjugative hyphae. Rarely a gamete so formed produces a parthenospore or a zygospore (2, 4). These parthenospores resemble small zygospores in appearance, and perhaps have been confused with them at times by other investigators. A careful investigation, however, will show that a perfect suspensor is present only on one side.

Races differing more or less from one another in growth appearance and even in spore characters have been found within many groups which we have considered single species. Our experience with both intraspecific and interspecific reactions leads us to believe that it is most convenient at least to consider races which form zygospores with each other as belonging to the same species. We have never ourselves found zygospore production between races obviously belonging to different species. BURGEFF, however, has obtained good crosses between mutant races of *Phycomyces* (7), and even between two distinct species of *Phycomyces* (8). Other reports of true hybridization among mucors must be accepted with caution, in view of the ease of confusing parthenospores with true zygospores. In general the production of progametes in intraspecific contrasts goes through

TABLE I

SOURCE OF RACES IN TABLE II

Species	Sex	Race	Source
Mucor H....................	+	......	Paradise nuts, New York City
Mucor dispersus.............	−	......	Leaves, Cold Spring Harbor
Mucor griseo-cyanus..........	+	......	Lab. culture, Cambridge, Mass.
Mucor griseo-cyanus..........	−	......	Centralstelle
Helicostylum piriforme........	+	863	Rat dung, Cold Spring Harbor
Helicostylum piriforme........	−	861	Brazil nuts, New York City
Choanephora cucurbitarum....	+	821	Squash, Cold Spring Harbor
Choanephora cucurbitarum....	−	832	Squash, Woodbury, L.I.
Mucor V....................	+	901	Horse dung, North Carolina
Mucor V....................	−	902	Horse dung, North Carolina
Rhizopus nigricans...........	+	899	Potato, Cambridge, Mass.
Rhizopus nigricans...........	−	900	Potato, Cambridge, Mass.
Circinella umbellata..........	+	897	Rat dung, China
Circinella umbellata..........	−	898	Dog dung, Cambridge, Mass.
Cunninghamella bertholletiae..	+	217	Brazil nuts, Oyster Bay
Cunninghamella bertholletiae..	−	266	Paradise nuts, New York City
Choanephora A.............	+	......	Raleigh, North Carolina
Choanephora A.............	−	......	Raleigh, North Carolina
Absidia glauca..............	+	915	Centralstelle
Absidia glauca..............	−	666	Soil, Cold Spring Harbor
Syncephalastrum racemosum..	+	651	Brazil nuts, New York City
Syncephalastrum racemosum..	−	652	Brazil nuts, Brooklyn, N.Y.
Cunninghamella elegans.......	+	496	Soil, Cold Spring Harbor
Cunninghamella elegans.......	−	506	Soil, Cold Spring Harbor
Mucor N...................	+	......	Cambridge, Mass.
Mucor N...................	−	......	Cambridge, Mass.
Mucor IV..................	+	921	Cold Spring Harbor
Mucor IV..................	−	922	Cold Spring Harbor
Absidia blakesleeana..........	+	369	Brazil nuts, Amsterdam, N.Y.
Absidia blakesleeana..........	−	571	Brazil nuts, Worcester, Mass.
Mucor III..................	+	......	Rat dung, Cambridge, Mass.
Mucor III..................	−	......	Rat dung, Cambridge, Mass.
Mucor VII..................	+	......	Guinea-pig dung
Mucor VII..................	−	......	Guinea-pig dung
Circinella spinosa	+	426	Brazil nuts, Worcester, Mass.
Circinella spinosa............	−	413	Brazil nuts, Huntington, L.I.
Mucor VIII.................	+	......	Hesperis, Cambridge, Mass.
Mucor VIII.................	−	......	Hesperis, Cambridge, Mass.
Absidia caerulea.............	+	533	Soil, Cold Spring Harbor
Absidia caerulea.............	−	912	Rabbit dung, Mt. Katahdin, Me.
Cunninghamella echinulata....	+	885	Lab. culture, Cambridge, Mass.
Cunninghamella echinulata....	−	886	Lab. culture, Cambridge, Mass.
Absidia cylindrospora.........	+	891	Centralstelle
Absidia cylindrospora.........	−	892	Centralstelle
Absidia repens..............	+	895	Brazil nuts
Absidia repens..............	−	896	Nuts, Margarita Island, Venezuela
Absidia whorled.............	+	395	Brazil nuts, Brooklyn, N.Y.
Absidia whorled.............	−	400	Brazil nuts, Storrs, Conn.
Phycomyces blakesleeanus	+	893	Rabbit dung, Cold Spring Harbor
Phycomyces blakesleeanus.....	−	894	Rabbit dung, North Carolina
Mucor VI..................	+	......	Horse dung, Cambridge, Mass.
Mucor VI..................	−	......	Horse dung, Cambridge, Mass.
Mucor mucedo..............	+	......	Horse dung, North Carolina
Mucor mucedo..............	−	......	Horse dung, North Carolina

to the formation of zygospores. Sometimes, however, as in certain contrasts of races of *Rhizopus*, many of the conjugations abort in various stages of development, leaving only a few perfect sexual spores. In *Cunninghamella bertholletiae* (4) certain combinations constantly gave only early stages of conjugation. Why this is so is not known, although in a few cases it was shown that perfect zygospore production could be induced in these cases by making the inoculations closer together.

The classification of races of mucors according to their sex by means of the imperfect hybridization reaction has been a common procedure in our laboratory. It seemed desirable, however, to study the reactions in more detail by making all possible combinations between selected mated pairs of races of the heterothallic species available.

The tests summarized in table II were made in 1919 and 1920, except those with *Muco mucedo* and *Mucor* VI, which are from earlier records. Races of these two species had become weakened or had died out at the time the series was tested. A few others of the species discussed in our preceding paper on intraspecific reactions are not included in the present study for similar reasons, or because the mated races were not available when the tests were made. Table I lists, with sources, the races according to the order in which they appear in table II. As may be seen by checking their numbers with the tabulations in our preceding paper, an attempt was usually made to use, in the interspecific tests, the strongest (+) and (−) races from each species as judged by their ability to take part in zygospore formation. *Mucor* H and *M. dispersus* are unmated (+) and (−) races which had been found especially valuable as imperfect hybridization testers. Since these experiments have been completed, the mate to *M. dispersus* has been found. Except for these two races, *Mucor* H and *M. dispersus*, the races of a given species are mated, that is, have produced zygospores when contrasted together.

The technique in making contrasts has already been described (6). The races to be contrasted were grown in pairs in watch glasses on nutrient 362 (2 per cent agar, 2 per cent whey powder, and 1 per cent dextrose), which seems to be the best medium for imperfect reactions of those tested, possibly in part because it does not support a too luxurious growth of aerial hyphae. Cultures were run in an oven

at a temperature around 24° C. *Mucor mucedo* and *Mucor* VI had been tested earlier under other conditions.

Since some species grow faster than others, there were four main groups established which were planted at 8 to 12 hour intervals. In the first group were the slowest growers, and these were planted

TABLE II

INTERSPECIFIC SEXUAL REACTIONS

(+) Races	(−) Races																											
	Mucor H	Mucor griseo-cyanus	Helicostylum piriforme	Choanephora cucurbitarum	Mucor V	Rhizopus nigricans	Circinella umbellata	Cunninghamella bertholletiae	Choanephora A	Absidia glauca	Syncephalastrum racemosum	Cunninghamella elegans	Mucor N	Mucor IV	Absidia blakesleeana	Mucor III	Mucor VII	Circinella spinosa	Mucor VIII	Absidia caerulea	Cunninghamella echinulata	Absidia cylindrospora	Absidia repens	Absidia whorled	Phycomyces blakesleeanus	AVERAGES	Mucor VI	Mucor mucedo
Mucor dispersus	A	B	B	B	C	B	O	C	C	D	C	C	C	C	B	D	C	C	O	C	C	C	O	O	O	1.80	..	..
Mucor griseo-cyanus	A	Z	A	A	O	C	C	C	B	O	B	C	B	C	O	C	O	O	D	O	C	C	O	O	O	1.58	..	..
Helicostylum piriforme	A	A	Z	A	C	A	B	A	A	D	C	A	C	B	C	C	B	B	B	C	O	D	O	O	O	2.38	..	..
Choanephora cucurbitarum	A	B	A	Z	D	C	B	B	B	D	D	O	C	B	D	B	B	B	O	O	C	C	O	O	O	1.83	..	..
Mucor V	A	A	C	B	Z	B	C	C	A	C	C	C	B	C	D	B	B	C	C	C	C	D	O	C	O	2.21	H	H
Rhizopus nigricans	A	B	B	C	D	Z	O	C	C	O	A	C	D	O	B	O	C	D	O	C	O	O	O	D	O	1.38	H	..
Circinella umbellata	A	B	C	B	O	O	Z	C	D	C	O	C	A	C	C	A	O	B	C	C	O	O	O	O	O	1.58	..	..
Cunninghamella bertholletiae	A	C	O	C	A	B	O	Z	C	B	C	C	C	C	O	O	C	O	C	O	C	D	O	O	O	1.46	..	..
Choanephora A	C	B	C	C	C	O	A	D	Z	O	O	O	O	C	D	C	C	B	O	C	O	O	O	C	O	1.25	..	..
Absidia glauca	A	A	D	B	C	A	C	A	D	Z	C	C	B	A	O	C	B	O	O	D	C	C	O	O	O	1.92	..	..
Syncephalastrum racemosum	A	B	C	D	C	A	O	B	B	D	Z	B	D	O	O	O	C	O	O	O	O	D	O	O	O	1.25	..	..
Cunninghamella elegans	B	A	O	O	C	C	O	C	O	O	D	Z	C	C	A	D	O	O	C	D	O	O	O	O	O	1.08	..	..
Mucor N	C	B	O	B	O	C	C	C	O	O	D	C	Z	C	O	O	C	D	O	O	O	O	O	O	O	0.92	H	..
Mucor IV	A	A	B	A	O	C	C	O	O	O	D	O	C	Z	O	O	O	O	O	O	O	O	O	O	O	0.92	..	..
Absidia blakesleeana	A	A	B	C	D	C	C	O	B	O	B	B	C	B	Z	O	O	O	O	D	O	O	O	O	O	1.38	..	..
Mucor III	C	A	A	B	O	O	A	D	B	O	O	B	O	O	O	Z	O	O	O	O	O	C	O	C	O	1.17	H	..
Mucor VII	A	B	B	A	O	O	B	O	B	O	O	O	O	O	O	O	Z	O	O	O	O	O	O	O	O	0.83	..	..
Circinella spinosa	A	A	B	C	O	C	C	O	O	O	O	C	C	O	O	O	O	Z	O	O	O	O	O	O	O	0.88	..	..
Mucor VIII	C	C	C	B	B	O	C	C	C	O	C	C	C	O	O	O	O	O	Z	O	C	O	O	O	O	1.08	..	..
Absidia caerulea	B	C	D	C	C	C	O	O	O	C	O	O	O	O	O	O	D	O	O	Z	O	C	O	D	O	0.75	H	..
Cunninghamella echinulata	A	B	D	C	C	C	O	C	C	O	C	O	O	O	O	O	O	O	O	O	Z	O	O	O	O	0.83	..	..
Absidia cylindrospora	B	C	O	C	O	O	O	C	O	D	O	O	O	O	O	C	O	O	O	D	O	Z	O	O	O	0.54	..	..
Absidia repens	B	C	D	O	O	O	C	D	O	C	D	O	B	C	O	O	O	O	O	D	O	O	Z	O	O	0.75	..	..
Absidia whorled	C	O	O	O	C	C	O	O	O	O	D	O	O	O	O	O	O	O	O	C	O	O	O	Z	O	0.38	..	..
Phycomyces blakesleeanus	C	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	Z	0.08	..	H
AVERAGES	3.36	2.88	1.75	2.25	1.16	1.71	1.46	1.54	1.58	0.67	1.25	1.38	1.50	1.29	0.71	0.92	1.04	0.75	0.50	0.79	0.58	0.67	0.00	0.33	0.00		..	..
Mucor VI	..	..	..	..	H	H	..	..	..	..	..	..	H	..	..	O	..	..	..	H	..	..	..	..	..		Z	H
Mucor mucedo	..	..	..	..	H	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	H		H	Z

first. These included *Absidia blakesleeana*, *Circinella spinosa*, *C. umbellata*, *Phycomyces*, and *Mucor dispersus*. Most of the species belong in the second group. *Cunninghamella bertholletiae*, *C. elegans*, and *Mucor* H comprised the third group. *Rhizopus*, *Cunninghamella echinulata*, and *Choanephora cucurbitarum* were in the fourth group, and, being the most rapid growers, were planted last. In cases in which growth was poor, or in which bacterial infection had occurred, the contrasts were repeated for the final record.

The contrasts were examined under the Greenough binocular two or three times at intervals determined by the amount of growth, the contact of the two races, etc. Contrasts, which by their last examination had not shown reactions, were further examined by teasing out bits of the interwoven hyphae of the two species under the compound microscope. By this latter more tedious method some strong and several weak reactions were disclosed which had not been apparent at previous examinations with the binocular, probably because of the occurrence of the reaction in these cases after the line of contact had become overgrown with aerial hyphae. After six to seven days zygospores were found in the control contrasts between (+) and (−) races of the species used as a tester. Parthenospores were formed in the contrast between "Whorled" *Absidia* and *Absidia caerulea*, and were not infrequent in combinations with *Mucor* H.

The strength of the imperfect reactions was graded from A to O, as was done in recording zygospore formation in our preceding paper. In making up table II, the grades of all the reactions with a given race were averaged by making A equal 4, and D equal 1, and the species were arranged according to the average rank of their paired (+) and (−) races. In making up the averages, the reactions (H) with *Mucor mucedo* and *Mucor* VI were not included. With the exception of these two species, all the possible 1225 combinations were made between the 25 pairs of (+) and (−) races. Sixteen contrasts are recorded involving *Mucor mucedo* or *Mucor* VI, making a total of 1241 combinations between races of 28 species included in 9 genera. Contrasts between races of like sex in all cases gave O reactions, and therefore are not recorded in the table. Since, as already pointed out, the grades of zygospore formation in different species are not comparable, perfect reactions between the selected pairs of a given species are not assigned grades in the table, but are indicated merely by the letter Z. Their relative sexual activity in zygospore production in comparison with other races of the same species may be seen from their grades in the preceding paper (5).

Table II shows that there is considerable difference in the strengths of imperfect hybridization. This difference seems to have no close connection with the taxonomic relationship of the species in question. The (+) strain, *Mucor* H, is the strongest of all, reacting with all the selected (−) races of the other species tested with grades

of C or better. Unfortunately no (−) *Mucor* H has yet been found. In general, in spite of a certain amount of irregularity, the races as arranged show the strongest imperfect reactions in the upper left-hand portion of the table, while in the opposite corner below the reactions are weak or zeros. The two members of a pair of races chosen for the test in many cases differ considerably in their ability to react with the selected races of other species. Thus the (−) race of *Absidia blakesleeana* has a grade for imperfect hybridization of 0.71, while its (+) race has a grade of 1.38. The two races have about the same grades for zygospore production (2.55 and 2.53). Likewise the (−) race of *A. glauca* with a grade of 0.65 is weaker than the (+) race of the same species with a grade of 1.92. It is possible that the races strongest in imperfect reactions would be found to average somewhat stronger also in zygospore production. The strength of reaction in the two processes, however, cannot be closely correlated. Thus *Helicostylum* ranks among the strongest species in producing imperfect reactions with other forms, but is one of the weakest in the production of zygospores. *Phycomyces*, on the other hand, is perhaps the most difficult species with which to induce the imperfect reaction, but gives zygospores in great abundance under the proper conditions of growth. External conditions apparently affect the processes differently. Thus it has been found that *Cunninghamella echinulata* will form zygospores only at temperatures above 20° C., but will enter into the imperfect hybridization reaction with other species at lower temperatures.

Rather extensive tests of the intraspecific sexual reaction leading to the formation of zygospores reported in our preceding paper have given no evidence for sex intergrades in the heterothallic races investigated. The present study of interspecific reactions, in which an imperfect reaction called "imperfect hybridization" is brought about, furnishes further evidence that sex intergrades in heterothallic mucors are at least extremely rare in nature, if not non-existent. The absence of sex intergrades in this group, in comparison with their frequency in dioecious flowering plants is perhaps connected with the fact that in the mucors we are considering gametophytes, whereas in the flowering plants we are considering sporophytes.

The establishing of a condition of strict sexual dimorphism in the forms studied, as well as their morphological simplicity of sex differ-

entiation, renders the group peculiarly adapted for use in an investigation of the fundamental differences between sexes. The fact that in heterothallic species all the races of one sex are theoretically capable of giving an imperfect reaction with all the sexually opposite races of every other species, while both sexes have been found to give imperfect sexual reactions with homothallic species (2), has led to the belief that there must be something fundamental common to all the (+) races, for example, responsible for these reactions. This belief was at the basis of the investigation now in progress (9, 10, 11), in which it has been shown that the (+) races of mucors correspond in biochemical behavior to the females of higher plants and animals, and the (−) to males.

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THE NATURE OF CHROMOSOMES

I. EFFECTS OF REAGENTS ON ROOT TIP SECTIONS OF VICIA FABA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 372

R. O. EARL

(WITH NINETEEN FIGURES)

Introduction

Since SUTTON (30), in 1902, first demonstrated the parallelism between the distribution of the chromosomes at meiosis and the incidence of hereditary characters, there has been a great and steadily increasing interest in the chromosomes. The work of MORGAN (23) and his associates and of many other investigators has demonstrated quite clearly that the chromosome theory of heredity rests upon a secure foundation of fact.

Among the decisive evidences may be mentioned chromosome aberrations, worked out genetically and cytologically, in the case of non-disjunction, by BRIDGES (3, 4), and afterwards developed by him in his studies on sex determination, in which he formulated his gene theory of sex; later induced artificially by MAVOR (21) by means of X-rays, and also brought about by many workers by means of species hybridization. Among these may be noted GOODSPEED and CLAUSEN (9), COLLINS and MANN (7), KIHARA (14), and SAX and GAINES (27). Chromosome aberrations have also occurred frequently in *Datura*, where they have been analyzed extensively by BLAKESLEE and BELLING (2). Other evidences are derived from sex-linked inheritance, the number and size of linkage groups, and the time and place in the life cycle of crossing over (PLOUGH 26).

All this evidence clearly shows that hereditary qualities are associated with particular chromosomes, and that chromosomes once lost are not regenerated, nor are the characters for which they were responsible regained. It goes farther. It places the gene theory, that heredity is due to material particles or genes arranged in linear order

in the chromosomes, on so secure a basis, and supports it with such a volume of quantitative evidence of the most precise nature, that it becomes of great importance that further cytological information about the chromosomes should be obtained.

The visible and readily stainable material, chromatin, of which the chromosomes appear to be mainly composed, at least at certain phases, is considered by the chemists to be relatively simple and comparatively uniform throughout living forms. MATHEWS (20) states:

> Chromatin apparently consists always of a salt of nucleic acid with a protein base. The nucleic acid is apparently the same, or at any rate closely similar in all the different cells examined; but the protein base is either a very basic, simple protein belonging to the group of protamins . . . . or it is a histon . . . . or it is a more complex and less basic protein of unknown nature in other nuclei.

One of the properties of chromatin is that it is very easily soluble in dilute sodium phosphate.

The chemical evidence thus appears to be distinctly unfavorable to the gene theory, which postulates a series of very unlike substances in the chromosomes, and not a material of uniform chemical composition. As MATHEWS says, "The foregoing discussion of the composition of chromatin . . . . lends no support to the hypothesis that the chromosomes are made of genes." We must therefore consider the evidence from cytology as to the physical nature of the chromosomes.

At present there are two main theories as to their physical constitution. According to one (GREGOIRE and WYGAERTS 10, SHARP 28) the chromosomes owe their different appearances at different stages in their life cycle to varying degrees of alveolation. The alveoli are filled with a non-staining substance, and when alveolation is most pronounced anastomosing strands of the staining substance or chromatin obscure the limits of the individual chromosome. No constant skeletal structure is recognized, and it is difficult to interpret the gene theory in its absence. If, however, the chromatin, which according to this school owes its threadlike appearance to the size and disposition of the alveoli, really has an intrinsic filiform nature of its own, we would still have much the same appearance as the proponents of the alveolation theory report. The significance of such a thread, however, might be very great.

It is this thread or chromonema hypothesis which forms the thesis of the other school. It has a long history, having been sponsored periodically since BARANETZKY (1) in 1880 reported a spiral thread in the chromosomes of several species of *Tradescantia*. It has been revived by the recent researches of MARTENS (18, 19), KAUFMANN (12, 13), KUWADA (15), and SHARP (29). All of these workers see a threadlike peripheral band or bands in the chromosome, and an interior substance which varies in staining capacity, and may interchange material with either the nucleolus or the karyolymph. The threadlike bands, or chromonemata, might conceivably carry the genes; and if they were to split lengthwise at each mitosis, as is maintained by some (notably KAUFMANN), the theory of the gene and this hypothesis would be in harmony, even if, as KAUFMANN maintains, the thread is double at all stages. If the number of threads in a chromosome remains constant, as is in fact claimed, it is immaterial how many there may be.

It is to be noted that MORGAN (22) has computed in three ways the maximum probable size of the gene in *Drosophila*, and has estimated its diameter as not exceeding 77 $\mu\mu$. Since the most minute particle which we may hope to render distinctly visible must have dimensions of at least 0.2 μ, it is apparent that particles of the preceding size are much below the limits of microscopic visibility. The number of genes in this insect has been estimated through the frequency with which the same point mutation occurs. This number has then been divided into the size of the sperm head, the combined size of the metaphase chromosomes, and the total estimated volume of the chromosomes at the synapsis stage. This gives the gene size as 77, 60, and 20 $\mu\mu$ respectively. The disparity here may be due partly to changes of volume due to fixation, but indicates more probably that the sperm nucleus and the metaphase chromosomes contain something besides genes. If this were true also for the synaptic chromosomes, the gene size on the basis of these computations would be further reduced. It is interesting to note that the size of a haemoglobin molecule has been estimated as 2.5 $\mu\mu$. While it is realized that no great reliance should be placed on these estimates of gene size, they are significant as an indication of possibilities in *Drosophila* and doubtless other forms as well.

The chemist considers chromatin to be a relatively simple homogeneous substance, varying little from form to form, and being most similar in animals and plants that are most closely related. Cytologists are agreed that the chromatin between mitoses forms anastomosing strands which grow out and connect with those of other chromosomes. The evidence of LEWIS and LEWIS (17) upon this point appears to be conclusive. KAUFMANN (12) reports a variation in the thickness and density of the chromonemata, and suggests that this is due to an interchange with the material of the nucleolus.

If the chromonema, or the chromatin, as displaced by alveoli, were to consist of an ultramicroscopic chain of genes associated with a varying amount of visible, homogeneous, readily staining material, we should have a situation in accord with the apparent morphology of the chromosomes and with the theory of the gene. It would also satisfy the chemist, who would realize that his analyses were of chromatin only, the genes being too small and too few of a kind to be distinguished by his methods, or to affect his determination of the constitution of chromatin.

Since it is notoriously difficult not only to observe accurately at very high magnification, but also impossible to be sure that material, after being killed, fixed, dehydrated, and stained, is still in its natural space arrangement, additional evidence from other sources is desirable.

Material and methods

As chromatin is readily soluble in dilute sodium phosphate, it was thought that it might prove profitable to observe the effects of this and other reagents upon sections of suitable material. Since *Vicia faba* has frequently been used for cytological research, notably by SHARP (28), and since root tips are readily obtained, this plant was chosen for study.

The comparative effects of various fixing agents were first examined. Chromoacetic-osmic mixtures appeared to produce the most natural effects, giving little or no evidence of distortion, and resulting in sharp staining of the chromatin when haematoxylin was used. Formalin-acetic-alcohol was also good. Absolute alcohol alone proved very unsatisfactory, causing much shrinkage of cell contents, and evident distortion. Carnoy's absolute alcohol-chloroform-acetic acid

formula gave much better results, but the finer chromatin threads often appeared irregularly coagulated and sometimes run together. No attempt was made to follow through the whole chromosome cycle, only critical phases being examined in detail.

It was considered that fixing agents containing compounds of heavy metals would prove unsatisfactory where the effects of reagents on sections were being tested, since stable salts of chromatin with these heavy metals would thus be formed. This was found later to be the case, and to apply also to mixtures containing formalin. Carnoy's fluid was therefore selected at the start, and, although other agents were tried, this proved the most satisfactory, using somewhat more acetic than indicated in the formula.

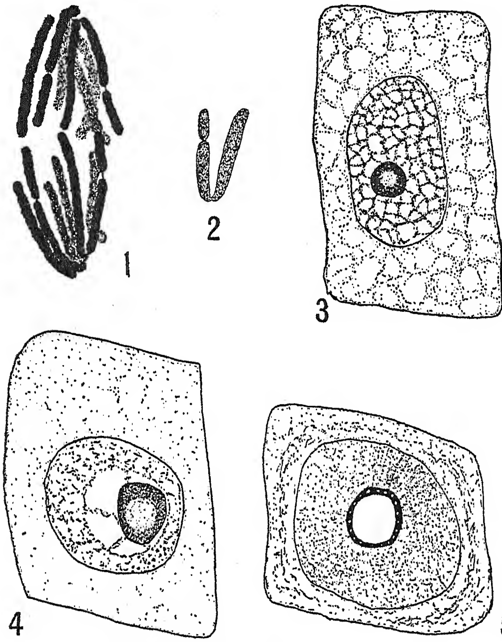
The material was imbedded in paraffin, and sections cut and mounted in the usual way. They were brought through xylol-alcohol and alcohol-water mixtures to water, when some slides were treated in aqueous solution of various reagents, while controls were stained directly. Solutions of Na_3PO_4 from 0.25 to 10 per cent, and similar strengths of NaH_2PO_4 were used. Some were then stained with safranin, a basic stain, some with phloxine, an acid stain, and others with iron-alum haematoxylin and orange G. The short method of mordaunting an hour and staining with haematoxylin an hour, as described by KAUFMANN (12), was used with good results.

In order to offset the effects of acidity or alkalinity of the reagent, a 2 per cent solution of NaH_2PO_4 was brought to pH 5 by titration with NaOH, using methyl red as an indicator. This H-ion concentration was selected because it was found by NAYLOR (25) to be near the isoelectric points of the materials of the cell. Other reagents also were used, including NaOH of the same alkalinity as 2 per cent Na_3PO_4 as determined by titration with phenolphthalein, and 1 per cent pepsin in 0.1 hydrochloric acid. Further, some slides after treatment with the basic phosphate were placed for some time in 0.1 N acid before staining.

Living root tips were also sectioned on the freezing microtome and the sections treated directly with various reagents. It was considered that cell permeability would be promoted by freezing, and thus the fresh protoplasm would be exposed directly to the reagent. Great difficulties were encountered in handling these sections, however, and the results obtained were inconclusive.

Results

The anaphase condition (fig. 1) in material fixed in chromo-acetic-osmic and stained in iron-alum haematoxylin by the short method shows some evidence of a double chromonema, as pictured by KAUF-

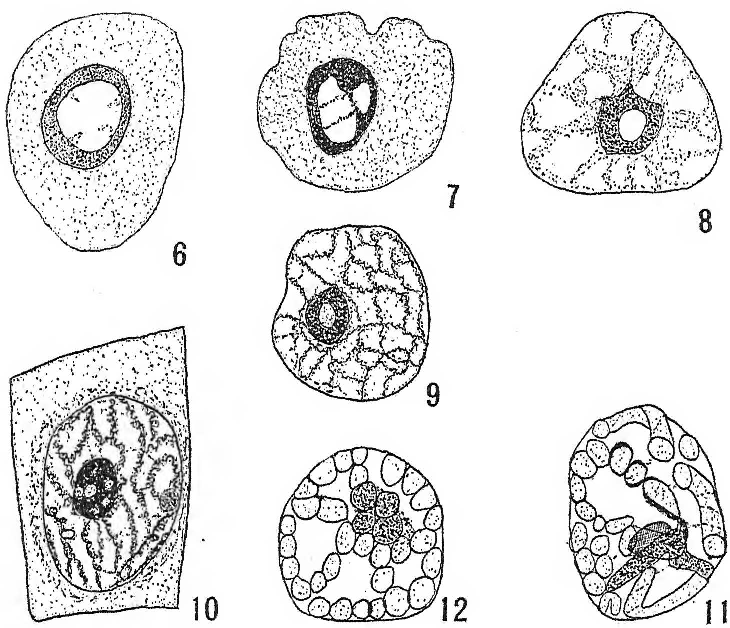


FIGS. 1–5*.—Fig. 1, anaphase chromosomes showing achromatic bridges; fixed in chromo-acetic-osmic solution and stained in iron-alum haematoxylin. Fig. 2, anaphase chromosome fixed in Carnoy's fluid and stained in iron-alum haematoxylin. Fig. 3, resting cell, chromo-acetic-osmic; iron-alum haematoxylin. Fig. 4, resting cell, Carnoy; iron-alum haematoxylin. Fig. 5, resting cell, Carnoy; 1 per cent Na_3PO_4; iron-alum haematoxylin.

* All figures were made at table level with the aid of a camera lucida on a Spencer microscope equipped with substage condenser, N.A. 1.40, Leitz 20×Periplan ocular, and Leitz 2 mm. apochromatic objective; magnification 2500.

MANN. Constrictions or achromatic bridges at points of spindle fiber attachment and elsewhere are visible, and also some indication of satellites. With Carnoy's fluid the appearance is very similar (fig. 2). The resting nucleus fixed in chromo-acetic-osmic (fig. 3) shows no positive detail. The nucleolus appears to be a double organ. With Carnoy's (fig. 4) the results are similar, but fixation does not appear to be so good.

When the sections are treated with Na_3PO_4 (0.5 per cent or stronger) before staining, no appreciable effects are to be observed if fixation has been in chromo-acetic-osmic or formalin-acetic alcohol;



FIGS. 6–12.—Fig. 6, nucleus only, fixed in absolute alcohol; 1 per cent Na_3PO_4; stained with phloxine. Fig. 7, nucleus only, Carnoy; 2 per cent Na_3PO_4 1 hour then 0.1 N H_2SO_4 one-half hour; stained in haematoxylin and orange G. Fig. 8, early prophase nucleus, Carnoy; 1 per cent Na_3PO_4; iron-alum haematoxylin and orange G. Fig. 9, prophase nucleus a little later than fig. 8 and treated like it. Fig. 10, prophase cell, later stage than fig. 9, same treatment. Fig. 11, prophase nucleus later than fig. 10, same treatment; nucleolus breaking down and appears to be flowing along spiral thread in chromosome; some chromosomes already formed where solution appears complete. Fig. 12, similar stage to fig. 11.

but with absolute alcohol or Carnoy's fluid as fixing agents there is a pronounced effect. The nucleus and chromosomes swell and lose their staining capacity, whether for acid or basic stains. The only exception to be noted is the nucleolus, where the peripheral region takes up the stain. With haematoxylin and orange G it colors light brown, with safranin and with phloxine, red.

In the so-called resting stage the region between the nucleolus

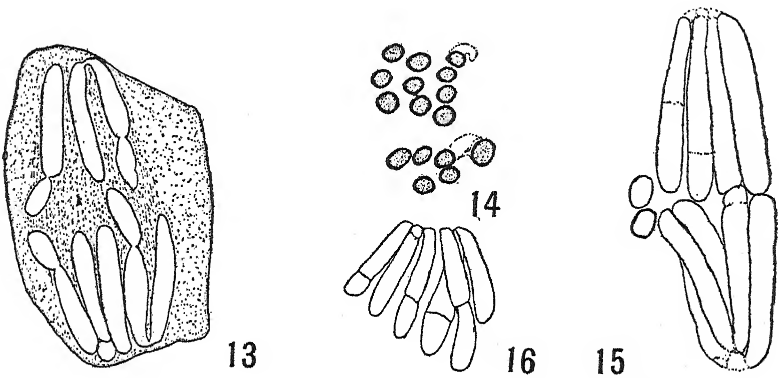
and the nuclear membrane has usually a radiate appearance, due to the process of swelling that has occurred (fig. 5). The cytoplasm is visibly crumpled by the expansion of the nucleus, often much more than is indicated in the drawing. Fig. 6 is a similar drawing of a nucleus only, but stained in phloxine instead of haematoxylin. The appearance of duality in the nucleolus is here pronounced. Fig. 7 is of a nucleus treated with 0.1 N H_2SO_4 after Na_3PO_4.

In early prophases evidence of some organization appears, but distortion is too great to permit of any interpretation (fig. 8). Later, however, reticulate units are seen (fig. 9) which appear to consist essentially of spirally twisted threads. In fig. 10 there is some evidence of a split in these threads, but the material stains so poorly that there could be no assurance on this point. The thread does not appear to be continuous. A still later stage (fig. 11) shows the organization of chromosomes in progress. Some of these appear to have taken definite shape, and resemble somewhat twisted cylinders with evidence of spiral markings. Others appear at first as an orderly arrangement of globules, but on closer examination, especially at some points, resemble more a cylindrical mass with spiral constrictions. It is as if a thread or threads were wound about this mass, which had swollen out through the meshes. The nucleolus, here stained brown due to haematoxylin and orange G, is partly disorganized, and appears to be flowing on to these threads and then throughout the chromosomes. Fig. 12 shows a less evident dispersal of the nucleolar matter, and an arrangement of the globular matter such that the spiral effect is not so pronounced.

Fig. 13 shows anaphase chromosomes. No indication of internal structure is visible, but the chromosome itself appears to be a homogeneous jelly-like mass inclosed in a definite skin or peripheral layer. Constrictions are still visible. Fig. 14, showing cross-sections of anaphase chromosomes, indicates this clearly, as do also the cut ends of two chromosomes in fig. 15. It is to be recalled that such structure was found by CHAMBERS (6) in his microdissection studies of chromosomes in living cells. That acidity does not affect the staining reaction is shown by figs. 14 and 16. These preparations are of anaphase chromosomes treated for one hour in Na_3PO_4, and afterwards with 0.1 N acid for half an hour before staining.

The telophase shows a mass of swollen chromosomes very tightly packed within a membrane. As in the anaphase condition, no internal chromosome structure is visible (fig. 17).

With pepsin hydrochloric acid no effect on the nucleus or chromosomes was observed. This is in accord with the work of ZACHARIAS (34), who treated living epidermal tissue and spermatozoa, and of JÖRGENSSEN (11), who used sections of alcohol-fixed animal egg. The sections came off the slide at first, owing to digestion of the egg

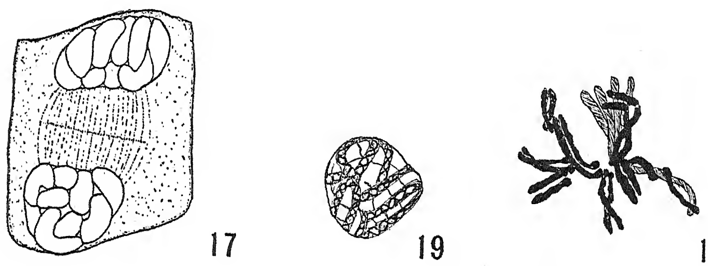


FIGS. 13–16.—Fig. 13, anaphase chromosomes, Carnoy; 1 per cent Na_3PO_4; iron-alum haematoxylin; chromatic material disappeared. Fig. 14, anaphase chromosomes in cross-section, Carnoy; 1 per cent Na_3PO_4 then 0.1 N HCl; iron-alum haematoxylin and orange G; peripheral layer of chromosomes apparent. Fig. 15, anaphase chromosomes, fixed in absolute alcohol; 1 per cent Na_3PO_4; phloxine. Fig. 16, anaphase chromosomes, Carnoy; 2 per cent Na_3PO_4 then 0.1 N H_2SO_4; iron-alum haematoxylin and orange G.

albumen. This was corrected by using a weak solution of potassium bichromate for floating out the paraffin ribbon on the slide, and then exposing the slides to light. The only apparent effect of the pepsin was the partial or complete digestion of the achromatic figure and the rendering more clear of the resting nucleus. This suggests that this figure may have some relation to the karyolymph, since both seem to be digested by the same enzyme. The chromosomes here stand out very clearly, especially in the metaphase. Fig. 18 shows part of an equatorial plate, where satellites and achromatic bridges will be observed, as well as the twisting about each other of the split halves, particularly noticeable in one case. This twisting is very pro-

nounced in earlier stages, and raises a query as to its significance that will be considered later.

A telophasic nucleus in pepsin-treated material is shown in fig. 19. Here the chromosomes stand out quite clearly, giving the impression of a cylindrical mass with a definite surface layer, within which lie two spirally intertwined chromatic threads. Connecting the loops and neighboring chromosome arms run anastomosing strands. Of course these chromosomes might be interpreted as vacuolate, but the impression one gets in focussing up and down is of spiral threads.



FIGS. 17–19.—Fig. 17, telophase, Carnoy; 1 per cent Na_3PO_4; iron-alum haematoxylin and orange G. Fig. 18, metaphase chromosomes, Carnoy; 1 per cent pepsin in 0.1 N HCl 2 hours; iron-alum haematoxylin and orange G. Fig. 19, telophase nucleus treated as for fig. 18.

With NaH_2PO_4 brought to pH_5 by titrating with NaOH, no appreciable effect was observed, except that the nucleolus stained a deeper black with haematoxylin than in untreated sections. The same result was obtained with NaH_2PO_4 alone in various concentrations from 1 to 10 per cent.

With NaOH of the same alkalinity as 2 per cent Na_3PO_4, results were obtained comparable with the effect of Na_3PO_4, but in less degree. The effects observed with the latter reagent are therefore not considered to be due to its alkalinity alone. MATHEWS (20) notes that chromatin is more soluble in dilute sodium phosphate than in sodium hydroxide.

Discussion

While the exposure of nucleus and chromosomes to reagents cannot be expected to give a natural picture of their constitution, yet

differential reactions of their constituents to these reagents would expose these differences which otherwise might not be apparent, and might throw some light on such problems as the genetic continuity of the chromosomes or of part of them, and the homogeneity or lack of it in the chromosomes themselves.

The resting nucleus when treated as described shows no sign of any regular structure whatsoever, but in the prophases an orderly arrangement comes into view. It is true that this arrangement may be variously interpreted. In terms of the chromonema hypothesis the constrictions visible in figs. 11 and 12 are due to the crossing of the dual threads, and this is more apparent in the preparations than can be drawn in two dimensions. But this involves two problems. A single row of these beadlike substances corresponds apparently to one chromosome. There is no evidence of a longitudinal split. Can it be that these two threads would later disentangle themselves and form a chromosome each? At present they appear to be definitely inclosed within the membrane of one chromosome. Or is each row only a split half of a chromosome? If so, the threads appear to be much farther separated than would be expected at this stage, on the basis of KAUFMANN's theory. Of course the swelling caused by the sodium phosphate might be expected to intensify the separation somewhat. I have not been able as yet to elucidate these matters.

The anaphase appearance might be considered due to the solution from the achromatic core of the chromatic element which is based upon the chromonemata; but if it dissolves entirely in the anaphase and telophase, why should the chromonema be visible in the prophase? This might be due to the circumstance that in the anaphase and preceding stages the chromonema is probably reinforced by and reacts with material from the nucleolus to form a substance soluble in sodium phosphate. Before this union both are insoluble in this reagent, and both appear in treated prophases. This view is reinforced by the findings of VAN CAMP (31), who worked with combinations of stains on nuclear material. He considers that the chromatic reticulum and the nucleolus form a special complex, "kinochromatin."

The nucleolus seems clearly to consist of two substances. This is evident in normal preparations and has long been recognized. It was

described by CHAMBERLAIN (5) in 1899, and is dealt with in detail in the most recent paper on the subject (LATTER 16, 1926).

There is nothing here that appears either to support or disprove the contention that the chromosome has essentially an alveolar structure. If the sodium phosphate removes the chromatin from a homogeneous achromatic core and the chromatin is the alveolated substance, we could thus account for the vacuolate appearance. Similarly the globular masses seen in the late prophase might be considered as large alveoli, the chromatin not being completely removed. This argument, however, is not so convincing as the chromonema hypothesis.

At any rate it seems clear that there is a substratum of achromatic material that has no orderly arrangement in the resting nucleus, but assumes a regular shape in the late prophase. It is also evident that the chromatic element is soluble in sodium phosphate, leaving no evidence of itself in the treated chromosome that is visible as such, and no anastomosing strands in the resting nucleus. These substances do not seem to have the orderly arrangement demanded of the physical basis of Mendelian heredity by the facts of genetics. The visible chromosome may even be thought of as an evanescent structure, being produced and then disappearing in each mitosis, as claimed by DELLA VALLE (8).

If we admit this assumption, however, we must account for the facts of the persistence of the chromosomes in developing the same sizes, shapes, and numbers in each mitosis, and also the persistence of loss or gain in numbers that occurs in chromosome aberrations. Might not these things justly be attributed to the influence of the ultramicroscopic thread of genes, of whose existence the geneticists have produced such a wealth of evidence?

These threads may be considered as placed axially, in which case neither alveoli nor chromonemata are of any great practical significance; but if they are conceived as the basis of the chromonema, we can imagine how they might function under these conditions. Depending on the fluctuating attraction of these threads for chromatin, the chromonemata would change in visibility as is claimed. If the attraction of certain genes or groups of genes for chromatin were greater than that of others, lumps of chromatin (chromomeres)

might appear on the chromonema. It is conceivable that in some forms these might be arranged in a regular way, and of course they would always appear the same in the same chromosome. WENRICHS' (32) well known work on *Phrynotettix* is a case in point.

This thread of genes would be the basis for the periodic building up and dissolution of the structures we know as chromosomes. Longitudinal splitting would be a function of the gene thread, occurring at a time when its attraction for chromatin is slight, thus making its visibility very low. The other materials which flow out in anastomosing strands at times, and at least appear to accumulate in the nucleolus and later flow back, must be homogeneous and therefore not divided in any precise or qualitative way at mitosis. The view that at least the chromatic material is not the physical basis of Mendelian heredity is gaining ground, and is expressed in two recent publications (LATTER 16, WILSON 33).

The twisting about each other of homologous chromosomes during meiosis and of split halves in mitosis, and the spiral twisting reported for the chromonemata, might all be related phenomena. If the genes are regarded as the basis of the chromonema, and considered as restricted to division at each mitosis in a plane parallel to the long axis of the chromosome, they may be thought of as dividing in every vertical plane in a haphazard way. Mutual repulsion of like products of division would tend to separate them as far as possible. But since the genes are regarded as arranged in linear order and presumably attached to each other in some way, since they maintain this arrangement, separation will not be a simple matter, the longitudinal split having occurred in various planes. On this account the two new threads would be thrown into the semblance of a spiral, although twists in one direction at certain places would largely be compensated by twists elsewhere the opposite way. This twisting with repulsion would greatly shorten the distance the threads cover, thus producing the chromosome as we know it. Moreover, since the spiral is then more apparent than real, disentangling of the threads at the next prophase would be relatively simple.

This proposal is based on KAUFMANN's claim that the daughter chromonemata are not found in separate chromosomes until a whole nuclear cycle after the split occurs. It may indeed explain why that

should be. If the split occurs in the prophase when the visible chromosome is formed with its tough peripheral layer, the split halves could not escape until after the next telophase, when the visible chromosome is dissolved; therefore we should find a dual thread in metaphase, anaphase, and telophase.

The twisting of homologous chromosomes, and of the newly formed products of division about each other, may perhaps be considered as a manifestation of gene attractions and repulsions at certain points on opposite chromosomes, thus simulating gene arrangement in a somewhat spiral way within the chromosome.

Finally, there is the problem of the functions of the visible parts of the chromosomes, if the ultramicroscopic thread is considered the essential part. Obviously they must form the internal environment of the genes. It is this material, or part of it, that the chemist has examined and found so stable and uniform, with but minor changes in protein constituent from one kind of plant or animal to another. The question arises as to what would happen to a form if a change were to occur in one of these protein constituents. Naturally the new internal environment would affect the reactions of the genes profoundly, and the more specialized the form the less likely would it be to survive the shock. The more simple forms would be better able to survive, but probably would show major changes in structure and function.

If we consider the course of evolution, we see that the great changes probably occurred long ago from relatively simple prototypes, followed by many minor changes of a specializing nature. The desmids and diatoms, the red algae, the mosses, the modern ferns, and many other such groups appear to be closed lines. Evolution is thus conceived tentatively as proceeding along two lines: great changes which are successful only in unspecialized forms, and minor changes involving specialization and rigidity. That the latter are gene mutations is indicated by the evidence of all such mutations whose origin is known. Not only do they involve special structures and functions, but nearly always they reduce the vigor of the plant or animal involved. As MULLER (24) states:

> Most mutations are deleterious in their effects. This applies not only to the organism as a whole, but also to the development of any particular part: the deli-

cate mechanisms for producing characters are more likely to be upset than strengthened, so that mutations should more often result in apparent losses or retrogressions than in "progressive" changes. This is both a priori expectation and a phenomenon generally observed.

The more disadvantageous mutations are eliminated through the failure of their possessors in the struggle of existence, although many such, if they are recessive, may persist in cross-bred forms. Others may survive if they can find or are subjected to peculiar environmental conditions to which they may thus seem to be adapted. The net result of gene mutations, therefore, is not so much deterioration as specialization.

As to the origin of the genes we have no information, but in the light of the nature of gene mutation the following quotation from MORGAN (23) seems significant:

> If the same number of genes is present in a white blood corpuscle as in all the other cells of the body that constitute a mammal, and if the former makes only an amoeba-like cell and the rest collectively a man, it scarcely seems necessary to postulate fewer genes for an amoeba or more for a man.

Summary

1. Sections of *Vicia faba* root tips were treated with solutions of tribasic sodium phosphate, acid sodium phosphate, sodium phosphate at pH 5, sodium hydroxide, and pepsin hydrochloric acid respectively.

2. Tribasic sodium phosphate and sodium hydroxide each caused a swelling of the nucleus and chromosomes, and almost eliminated their staining capacity. The effect of the former reagent is the greater. It is considered that the chromatin is dissolved and removed.

3. The nucleolus is shown to consist of two elements, a peripheral and central, the former probably contributing to the formation of the chromosomes.

4. A theory is proposed to account for chromonemata as indicating the presence of a thread of ultramicroscopic genes whose split halves mutually repel each other within the chromosome.

5. The suggestion is made that the visible parts of the chromosome and nucleus form an internal environment for the interaction of the genes, but are not themselves the physical basis of Mendelian heredity.

6. Changes in the constitution of this visible matter might produce a profound effect that only unspecialized forms could survive, gene mutations being responsible for minor changes involving specialization.

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VARIATION IN SUGAR CONTENT IN POTATO TUBERS CAUSED BY WOUNDING AND ITS POSSIBLE RELATION TO RESPIRATION[1]

E. F. Hopkins

(WITH FOUR FIGURES)

While investigating the effect of low temperatures on respiration and carbohydrate changes in potato tubers, it was found by chance that wounding of potato tubers causes a marked change in the sugar content in a short period of time. The particular experiment which showed this was being carried out to gain some idea of the amount of variation to be expected in the chemical analyses due to sampling.

In preparing four samples of ten tubers each for chemical analysis, two cylinders were cut from each tuber with a cork borer, to be used for catalase determinations. The remainder of the same in each case was ground to a pulp and sampled for sugar analysis and moisture determinations. Due to lack of time it was not possible to grind these samples promptly, and therefore several of them were held for a time with cylinders cut from them (that is, in a wounded condition) before being sampled. Sample I was ground for analysis early in the afternoon shortly after wounding; sample II was ground late this same afternoon; sample III the next morning; and sample IV the afternoon of the second day. The results of the sugar analyses and moisture determinations are shown in table I.

Experiment I

The results of the sugar determinations on the wet basis are seen to increase progressively from sample I to sample IV. That this increase is not due to their increase in total solids caused by drying is evident when the data are calculated to the dry basis. In approximately one day the sugar content has increased from 2.302 to 3.400 per cent, according to the dry basis. The experiment, therefore,

[1] This investigation was carried out at Cornell University under a National Research Fellowship in the Biological Sciences.

while giving no data on error in sampling, because of delay in preparing samples after wounding, does bring out the interesting information that there is marked increase in sugar content in potato tubers following wounding.

It is well known that wounding causes an increase in the rate of respiration. This effect was first observed by BÖHM (2), and was later confirmed by STICH (17), who brought out that if the cut surfaces of the tubers were sealed together with neutral gelatin, immediately after wounding, the increase in respiration was not so great. RICHARDS (15) studied the effect of wounding on respiration more extensively, and has shown that an increase in respiration follows

TABLE I

INCREASE IN SUGAR CONTENT AFTER WOUNDING

SAMPLE	PERCENTAGE SOLIDS	PERCENTAGE SUGAR, WET BASIS		PERCENTAGE SUGAR, DRY BASIS	
		Reducing	Total	Reducing	Total
I....................	20.79	0.169	0.478	0.812	2.302
II....................	21.87	0.147	0.520	0.673	2.379
III....................	24.59	0.185	0.648	0.752	2.636
IV....................	23.60	0.261	0.802	1.105	3.400

wounding in potato tubers as well as in fleshy organs of other plants. In general he obtained a respiration-time curve which rises rapidly to a maximum the second hour after wounding, declines, and rises more gradually to a second maximum at the end of about 24–30 hours. There is a gradual decline from this second maximum.

It occurred to the writer, after obtaining these results, that it would be of interest to determine whether the increased respiration obtained by RICHARDS could be correlated with these changes in sugar content. Various investigators have found that the addition of sugar to culture media which come in contact with the material concerned or in which plants are grown influences respiration markedly. KOSINSKI (6) showed for *Aspergillus niger* a striking effect of sugar on respiration. When he washed out the nutrient solution in which this fungus was grown with the same nutrient solution minus sugar, but made isotonic by the addition of NaCl, respiration fell to a low

value. On the addition of the normal nutrient it rose again even higher than the original value. PALLADIN and KOMLEFF (12) found that etiolated bean leaves left with the petioles in sugar solutions respired much more rapidly when transferred to pure water. PURIEVITCH (14) demonstrated in the case of *A. niger* that as the concentration of sucrose increases from 1 to 25 per cent, the respiratory ratio $\frac{CO_2}{O_2}$ passes through a maximum when the sugar concentration is about 10 per cent. Perhaps the most complete paper on the effect of sugar on respiration is that of MAIGE and NICOLAS (9), who have determined the effect of varying the concentration of a considerable number of sugars on respiration. They have shown that, in general, as the concentration of the sugar in the medium increases respiration increases, until very high concentrations are reached, when partial plasmolysis occurs and respiration drops. KNUDSON (5), in his work on the effect of carbohydrates on green plants in pure culture, showed that the addition of maltose, glucose, or sucrose increased the amount of carbon dioxide markedly over that produced by the checks to which no sugar was added. He states that while this is partly due to the greater root development, it is also due to a higher rate of respiration. In these papers the internal concentration of sugar was not determined, although MAIGE and NICOLAS rightly point out that the cellular concentration of sugar is the important factor concerned with respiration, not the amount added to the culture medium.

In the case of the potato tuber we have an organism in which the cellular concentration of sugar may change either by hydrolysis of starch to sugar or by synthesis of starch from sugar, these changes being brought about by various causes. Hydrolysis of starch to sugar is brought about by keeping the tubers at 0° C., and MÜLLER-THURGAU (10) explains the increased respiration of tubers previously held at 0° C. over those held at a higher temperature by this increased sugar content. The writer also believes that this explains the results of his own experiments (3), in which the respiration at 0° was found to be greater than at 4° C.

As it appeared from the preceding experiments that wounding potato tubers also increases their sugar content, other experiments

were performed to confirm the data, and to correlate if possible the changes in sugar concentration with the changes in respiration obtained by RICHARDS.

Experiment II

A number of rather small tubers were selected and kept at laboratory temperature, which varied from 20°–22° C., for several days. The respiration of six of these tubers was determined. The next day the respiration of six other tubers was determined, and these tubers were then reduced to a pulp and the latter sampled for sugar analysis and for moisture determinations. At this time the rest of the potatoes were wounded by cutting two cylindrical plugs from each. At certain intervals samples of six of the wounded tubers were placed in the respiration chamber and their respiration determined. They were then sampled for analysis as in the case of the unwounded tubers.

The respiration apparatus used was similar in principle to that used by the writer in previous work on the effect of low temperatures on respiration (3), but on a smaller scale. The respiration chamber consists of a one-quart fruit jar fitted with a no. 12 stopper, through which pass inlet and outlet tubes. The absorption unit is made up of a 500 cc. Erlenmeyer flask, fitted with a rubber stopper through which pass an inlet and an absorption tube. This is a 50 cc. burette tube containing beads in the lower portion, and fitted with a small rubber stopper and outlet tube. The apparatus is guarded from atmospheric carbon dioxide by means of a soda-lime tower. Barium hydrate solution tubes are inserted before the respiration chamber and after the absorption unit, as precautionary tests. In none of the runs was there noted the entrance of atmospheric carbon dioxide or the loss of carbon dioxide respired, through the absorption tower. The current of air was produced by a Richards pump. During this experiment the respiration chamber was immersed in a water bath kept at 20° C. Before each run the precaution was taken to aerate the apparatus with carbon dioxide-free air.

The carbon dioxide was absorbed in 25 cc. of N/10 NaOH solution, to which had been added 5 cc. of a solution which contained 1 gm. of $BaCl_2$. At the conclusion of a run the solution in the tower was allowed to drain into the flask, and the tower rinsed with carbon

dioxide-free water which was allowed to drain into the flask. The excess of alkali was then titrated in the same flask with N/10 HCl in the presence of ortho-cresol phthalein. As in previous work, a blank was run on the alkali plus the $BaCl_2$. The difference between the blank and the titration after absorption represents the carbon dioxide absorbed in terms of N/10 acid. The end points in the titrations were accurate to one drop of N/10 acid.

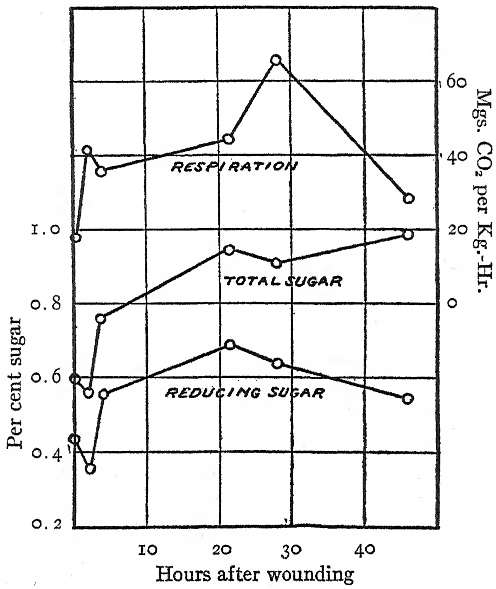

FIG. 1.—Changes in respiration, total sugar, and reducing sugar after wounding; experiment II.

The sugar analyses and moisture determinations were carried out as previously described (3).

The results obtained in experiment II are summarized in table II. It will be noted that there is good agreement in the respiration data on two different lots of tubers before wounding. The gradual increase in total solids is due to evaporation from the cut surfaces in the somewhat dry atmosphere. The data are shown graphically in fig. 1. The respiration curve is in general the same as those obtained by RICHARDS for potatoes. The marked increase in both reducing

sugars and total sugar found in experiment I was again noted. The reducing sugar appears to reach a maximum at the end of about 2

TABLE II

EFFECT OF WOUNDING ON RESPIRATION AND SUGAR CONCENTRATION IN POTATO TUBERS

DATE	HOUR	HOURS AFTER WOUNDING	WEIGHT OF TUBERS (GM.)	RESPIRATION MG. CO_2 PER KG PER HOUR	SUGAR CONCENTRATION PERCENTAGE, MOIST BASIS		PERCENTAGE SOLIDS
					Reducing	Total	
11-5-24	4:56 P.M.	0	457	18.4	....	....	....
6-24	11:35 A.M.	0	420	18.7	0.440	0.600	23.87
	2:10 P.M.	2	336	41.7	0.358	0.566	23.22
	4:12 P.M.	4	347.5	36.1	0.562	0.768	23.71
	7:24 A.M.	21.5	300	44.7	0.693	0.957	24.33
	1:54 P.M.	26	255	54.4	....	....	....
	3:54 P.M.	28	300	66.1	0.642	0.912	25.20
8-24	10:15 A.M.	46	322	29.0	0.558	0.995	25.94

hours, and then falls off, but the total sugar at the end of the experiment was still increasing. In general the results show that the increase in wounding is accompanied by an increase in sugar content, and that these two phenomena are approximately correlated.

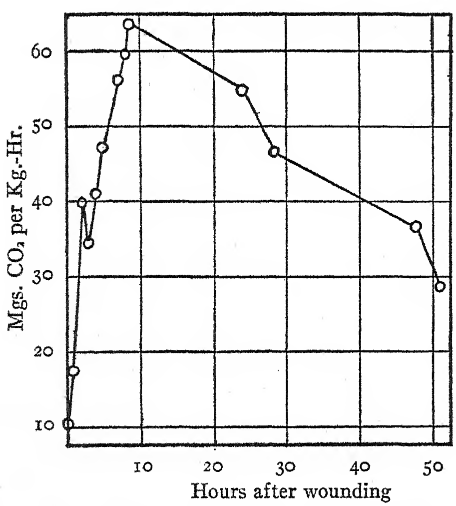

FIG. 2.—Changes in respiration after wounding; experiment III.

Experiment III

In this experiment only the respiration was studied. Two runs were made on the normal tubers, and then a series of determinations of CO_2 carried out on the same material after wounding. The data are shown in table III. Again these results are similar to those of RICHARDS, with the second maximum, however, falling probably between eight and twenty-four hours after wounding. The first maximum ap-

pears as before at the end of the second hour. The results for the first fifty-one hours are shown graphically in fig. 2.

TABLE III

EFFECT OF WOUNDING ON RESPIRATION OF POTATO TUBERS

DATE	HOUR	HOURS AFTER WOUNDING	RESPIRATION MG. PER KG PER HOUR
11–13–24	11:00 A.M.	0	9.54
	12:08 P.M.	0	10.47
	1:21	1	17.74
	2:21	2	39.95
	3:31	3	34.61
	4:21	4	41.18
	5:21	5	47.40
	7:21	7	56.36
	8:21	8	59.68
	8:51	8½	63.86
11–14–24	12:01	24	55.02
	4:51	28½	46.82
11–15–24	12:30	48	36.75
	3:15	51	28.61
11–18–24	12:21	120	25.94

TABLE IV

EFFECT OF WOUNDING ON RESPIRATION

DATE	HOUR	HOURS AFTER WOUNDING	RESPIRATION CO_2 MG. PER KG HOUR	DATE	HOUR	HOURS AFTER WOUNDING	RESPIRATION CO_2 MG. PER KG HOUR
12–1–24	12:19 P.M.	0	16.0	12–2–24	8:00 P.M.	13	35.1
	2:19	0	16.5		9:00	14	38.9
	4:19	0	16.2	12–3–24	10:05 A.M.	26	40.8
12–2–24	8:25 A.M.	½	40.7		11:05	27	42.5
	8:55	1	42.6		12:05 P.M.	28	40.1
	9:25	1½	47.4		2:30	30½	42.4
	9:55	2	34.3	12–4–24	4:30	32½	39.4
	10:25	2½	44.1	12–5–24	4:30	56	38.7
	10:55	3	46.4		12:05	76	33.0
	11:25	3½	45.3		2:05	78	34.4
	12:25	4½	46.8	12–6–24	4:05	80	33.6
	2:25 P.M.	6½	46.7		12:00 M.	100	28.3
	7:00	12	43.9				

Experiment IV

This experiment was carried out as experiment II, with the following exceptions. The respiration apparatus was placed in the chamber of an incubator where the temperature was maintained constant at 25° C. The wounded tubers for analysis were placed in a

large desiccator in the same incubator, and aerated by means of a current of moist air to prevent their drying out. The same wounded sample was used throughout the experiment for respiration deter-

TABLE V

EFFECT OF WOUNDING ON SUGAR CONTENT OF POTATO TUBER

Date	Hour	Hours after wounding	Percentage sugar, moist basis	
			Reducing	Total
12-1-24	....	0	0.261	0.458
12-2-24	8:45 A.M.	1	0.314	0.522
	11:20	$3\frac{1}{2}$	0.374	0.563
	2:17	$6\frac{1}{2}$	0.235	0.406
	4:07	$8\frac{1}{3}$	0.356	0.566
	7:40	12	0.298	0.502
12-3-24	9:45 A.M.	26	0.258	0.513
12-4-24	10:20	$50\frac{1}{2}$	0.334	0.638
12-6-24	10:00	99	0.427	0.699
12-8-24	1:35 P.M.	151	0.306	0.581

minations, and samples were taken from the desiccator for analysis at more frequent intervals. By continuing the experiment over a longer time, the approximate time of maximum sugar content was also determined. The respiration data are presented in table IV, and the result of sugar analysis in table V.

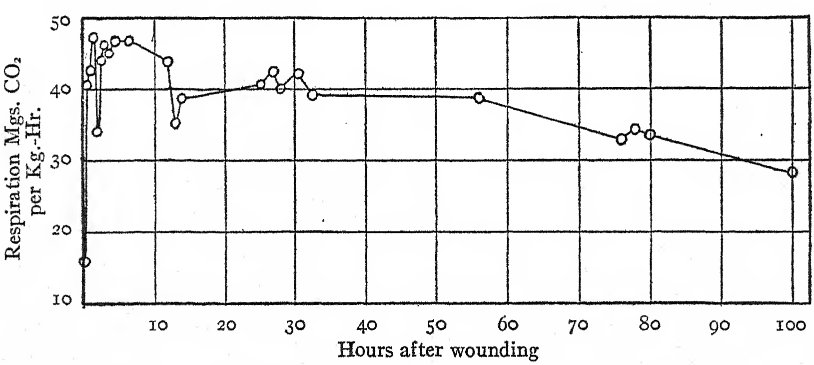

FIG. 3.—Changes in respiration after wounding; experiment IV

From table IV it will be observed that the respiration, very constant before wounding, rises rapidly as soon as wounding takes place, reaching a maximum at the end of one and one half hours; it drops off

at the end of the second hour but rises rapidly again and then gradually falls off. Fig. 3 shows the general shape of the curve. The second maximum in this case occurs even somewhat earlier than in the foregoing experiment. The curve, however, is somewhat flattened.

The data for the sugar analyses are plotted in fig. 4. It is seen that the curves also show a double maxima, the first three to six hours after wounding, and the second four days. The curves for reducing and total sugar parallel each other throughout.

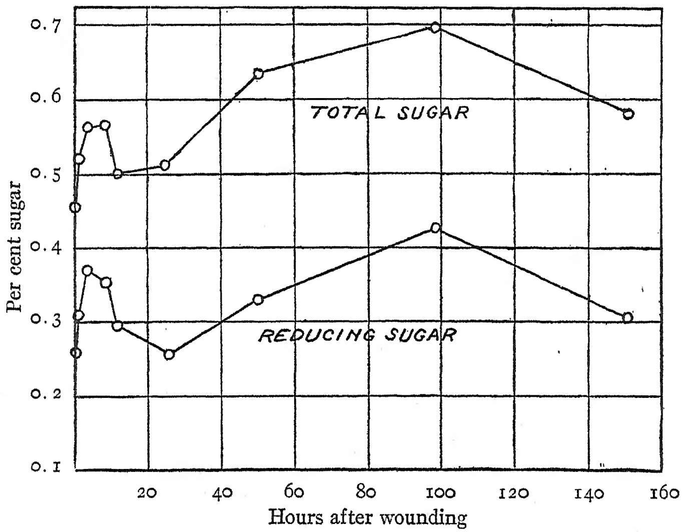

FIG. 4.—Changes in total reducing sugars after wounding; experiment IV

Discussion

From the experimental data presented there is no doubt that the sugar content of the potato increases on wounding. Considering only the total sugar, the maximum increase in experiment I is 68 per cent, in experiment II 66 per cent, in experiment IV 53 per cent. The exact course of this sugar concentration change the writer believes would have to be ascertained by a greater number of experiments; in fact, it probably will vary with the variety and condition of the tubers and possibly with the relative humidity under which they are kept after wounding. According to APPEL (1), suberization is more rapid if the wounded tubers are kept in a humid atmosphere, and, as

the writer hopes to bring out in this discussion, sugar formation after wounding is related to suberization and cork formation. The data for experiment IV, however, show a definite trend and are suggestive.

The increase in sugar concentration is associated no doubt with the general activities brought about in the tubers by wounding, which lead to the formation of suberin on the cut surfaces and to the formation of a new meristem from the parenchyma in this region. PRIESTLY and WOFFENDEN (13) have discussed the factors concerned in the formation of this cork layer. During the course of one experiment the writer sectioned some of the material which had been wounded about five days previously, and observed a clear area in the tissue extending about 2 mm. in from the wounded surface. Microscopical study showed the cells in this region, usually packed with starch, were practically free from it. The meristem as described by PRIESTLY and WOFFENDEN was evident in about the center of this clear area, no starch being observed between the suberin and the meristem. On the extreme outer surface beyond the suberin layer some starch grains were noted, which had possibly been cut off from the diastatic action by the layer of suberin. It was interesting to observe the details of cell structure in cells free from the starch which usually obscures them. In many cells the nucleus and nucleolus stood out clearly, and delicate radiating strands of cytoplasm were seen; in other cells a beautiful foamlike structure of the cytoplasm was present. In a recent paper NAKANO (11) also mentions the absence of starch in this area adjacent to the wounded surface.

One would expect, therefore, to find after wounding a higher concentration of sugar near the wounded surface, than in the tuber as a whole. It will be recalled that the analyses reported are on the basis of the tubers from which two cylindrical plugs had been removed. In order to test this idea, some of the cylindrical plugs cut from the tubers in experiment IV were kept and analyzed after several days. These plugs, having a greater proportion of wounded surface per unit volume of tissue, should have a greater sugar content than wounded tubers sampled for analysis at the same time. They had in fact a higher content of both reducing and total sugar than any of the wounded tuber samples. The results are given in table VI.

In this connection an experiment of SCHNEIDER-ORELLI (16) is pertinent. He made a qualitative test with Fehling's solution on

tubers which had been wounded four days previously, and obtained a much stronger test in this clear region near the wounded surface.

As brought out previously, there is a correlation between cellular sugar concentration and respiration, and in the case of wounding one would expect that the increased sugar content would cause an increase in respiration of wounded potato tubers. One objection to this view, of course, would be the fact that RICHARDS in his experiment obtained increases in respiration on wounding beets and other storage organs, which have no starch reserve as in the potato. If one examines the data in these instances it will be noted that the increases are

TABLE VI

SHOWING GREATER INCREASE IN SUGAR CONTENT NEAR WOUNDED SURFACE

	PERCENTAGE SUGAR	PERCENTAGE INCREASE OVER UNWOUNDED TUBERS
Cylinders		
Reducing sugar	0.486	86
Total sugar	0.735	60
Wounded tubers		
Reducing sugar	0.427	64
Total sugar	0.699	52

not so large, and the second respiration maximum is not so marked. It is entirely possible that here the increased respiration is due entirely to the changes brought about by the diminution in the carbon dioxide concentration in the tissues. MAGNESS and DIEHL (8) have shown that coating apples with paraffin or oil causes a reduced respiration rate. At the same time they show, by analysis of the intercellular air, that while there is sufficient oxygen, carbon dioxide accumulates. They ascribe the reduced respiration rate to this accumulation of CO_2. Conversely one might assume that a diminution of the CO_2 concentration would accelerate respiration. RICHARDS himself states that the results were not so striking as in the experiments with potatoes and carrots. On the other hand, one cannot entirely account for the increase in respiration in potatoes on this basis, when those experiments of RICHARDS are considered where the wounded parts were immediately stuck together by means of clay after being cut. In these cases the initial maxima were absent, but the secondary

maxima, although not so great, were very marked. Here the sugar produced in the process of wound healing is probably a factor.

In one of RICHARDS' experiments, for example, the respiration of 225 gm. of potato tubers was determined and found to be less than 1 mg. per hour. They were then halved and immediately stuck together again with neutral clay. No preliminary maximum occurred, which is in accord with RICHARDS' idea that there is no rapid outward diffusion of CO_2; but the respiration gradually increased, until at the end of 24 hours the rate was 7.5 mg. per hour. At the end of 55 hours it was 2.5 mg. per hour. While this is by no means a large increase, yet compared with other experiments in which the cut parts were not stuck together with clay it is a large increase, probably at the greatest being more than eight times the rate before wounding.

It should be pointed out that, when the cut parts are stuck together with neutral clay (as in the experiments of RICHARDS), although the air is excluded and suberization does not occur, meristem or cork cambium formation takes place (13), with the accompanying hydrolysis of starch to sugars.

MAGNESS (7) has suggested that part of the increased respiration following wounding is due to a mechanical facilitation of the gaseous exchange and part to metabolic changes in the wounded tissue. JOHNSTONE (4) concludes, from an experiment of STICH (17) on potato tubers, that 120.9 per cent of the increase obtained in the rate after wounding is due to injury, and 376.7 per cent is due to the facilitation of gaseous exchange. In his own experiments with sweet potatoes, he found 97.3 per cent increase in respiration when cut parts were exposed, and 17.15 per cent when they were immediately sealed together after cutting.

When measuring respiration by means of gas exchange, it should be pointed out that other factors should be considered in interpreting results. For instance, as soon as wounding occurs, processes are set in action which produce suberization of the wounded surfaces, and thus tend immediately to cut down gaseous exchange. Even if we postulate a direct effect of sugar concentration on respiration, therefore, we must expect the maximum in sugar concentration to lag behind the point of maximum respiration. At the same time that the sugar content is causing an increase in the respiration, suberization of the wound is cutting down gaseous exchange, causing an increase

in the amount of carbon dioxide in the tissues, which has been shown to lower the respiratory rate (8). On this basis the explanation of the results obtained in experiments II and IV is obvious. In both of these experiments the total sugar curve is still rising after the maximum respiration point has been passed.

Perhaps too close a correlation should not be drawn from the limited data presented, but in general it is true that with increased sugar the respiration is augmented. Even after considerable time has elapsed following wounding and callus formation, respiration is still much greater than before wounding, both in my experiments and in those of RICHARDS. At the time the sugar content is still higher than in the unwounded state.

In experiment IV the preliminary maximum at the end of a few hours is interesting, and may be caused by a disturbance of equilibrium conditions in metabolism, brought about by the sudden release of carbon dioxide from the tissues which RICHARDS has shown to take place when the tubers are first cut.

Summary

1. A marked increase in sugar content is shown to follow wounding. The maximum increase found in the experiments reported varied from 53 to 68 per cent of the original sugar content.

2. In general the sugar content rises to a maximum after wounding, reaching a high point after several days, and again falls off. It is possible that there is a preliminary maximum not so great as the other at the end of a few hours.

3. From microscopical observations and chemical analyses this increase in sugar content is thought to be brought about by activities leading to callus formation.

4. The augmentation of respiration which follows wounding can be explained logically on the basis of the increase in the sugar content of the cells, although it is pointed out that because of other factors the curves for respiration and sugar content will not be exactly parallel.

LABORATORY OF PLANT PHYSIOLOGY
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ABNORMAL SEX ORGANS OF MNIUM MEDIUM

George S. Bryan

(WITH TWENTY FIGURES)

Introduction

The discovery of numerous examples of abnormal sex organs in *Mnium medium* has made it seem worth while to investigate carefully the details of these peculiar structures.

Lindberg (5) has reported that in 1878, while examining samples of female plants of *Hypnum*, certain perichaetia were noted which, in respect to the length and breadth of their leaves, stood midway between male and female perichaetia. On closer inspection sex organs were found, some of which resembled archegonia in structure, while others bore a closer resemblance to antheridia, so that one might adduce a series of transformations from actual female to actual male organs. The venter of a transformed archegonium was observed filled with extremely fine grained material, perfectly resembling a dried mass of spermatozoids; but Lindberg was unwilling to commit himself that this dried mass was actually equivalent to such. No rounded egg could be seen in the venter of any transformed organ. A plate of drawings showing the external appearance of these organs accompanies the article.

Hy (4), in a paper on the archegonium of the mosses, mentions that different species of mosses have archegonia transformed at their summit into antheridia. He states that *Atrichum undulatum* is one of the species in which this phenomenon is most frequent, especially in the synoicous inflorescences terminating a stem two years old. No figures are given to illustrate the statements.

De Bergevin (2) has reported specimens of *Plagiothecium sylvaticum* with marked peculiarities in the forms of the sex organs. These are borne in groups along the lower portions of the branches of a plant. For the most part these groups were composed of antheridia only, but each of a few groups consisted of an archegonium surround-

ed by two to several antheridia. In some groups DE BERGEVIN found what he termed archegonia being transformed into antheridia: "En réalité, il y a là une véritable interversion dans le process organique." A series of sketches showing in outline form these transformations from archegonia to antheridia accompanies the article. In explanation of this phenomenon of transformation, DE BERGEVIN advances the hypothesis that shade and moisture are the controlling factors. When the conditions of shade and humidity remain below a certain limit only archegonia are produced; when this limit is exceeded archegonia give place to antheridia. The intermediate condition favors synoicous plants. DE BERGEVIN is silent on the question of the actual formation of antherozoids in the transformed antheridia. He merely asks the question, "Les anthéridies anormales sont elles capables de féconder l'organe femelle?"

HOLFERTY (3), in an account of the development of the archegonium of *Mnium cuspidatum*, has briefly described a number of abnormal organs that "constitute a progressive series leading from the normal archegonium to the normal antheridium." Several drawings illustrate the structure of these organs as seen in section. HOLFERTY speaks of sperm mother cells as being present in one of the organs, but does not discuss the actual formation of antherozoids.

Shortly after the appearance of HOLFERTY's paper, LYON (6), in an article on the evolution of sex organs in plants, makes brief mention of HOLFERTY's paper, and adds that "specimens have been secured with perfectly typical moss sperms which were discharged from the antheridial region above the egg."

Material and methods

The material for this study was gathered for several years in succession from a bank having a northern exposure on the shore of Lake Mendota, near Eagle Heights, and was supplemented by collections from the Dells of the Wisconsin River. The plants from both localities have been identified by Mrs. ELIZABETH G. BRITTON as *Mnium medium* (Bryol. Eur.). It is interesting to note that the material from these two stations, approximately 60 miles apart (the only localities in which thus far I have been able to find *M. medium*), showed about equal numbers of abnormal organs. Since these ab-

normal organs have regularly recurred for a number of years in both of these stations, it seems probable that they are a constant feature of *M. medium*.

Various killing agents were used, but the most satisfactory results were obtained with Flemming's medium, and with Rawlins' formal-acetic-alcohol. After imbedding in paraffin, serial microtome sections were cut 5–12 μ in thickness. As stains, safranin in combination with Licht Grün, and Flemming's triple were employed. Considerable difficulty has been experienced in sectioning mature plants, since the usual process of imbedding renders them hard and brittle.

Development of normal sex organs

Mnium medium is synoicous. Many hundreds of heads have been studied, and thus far no exception to this statement has been found.

In the spring of the year, among the old branches, two kinds of new branches make their appearance. The purely vegetative branches grow long and slender, have a small bud at the apex of the branch, and quickly tend to assume a dorsiventral position. The gametophores, on the contrary, are short and stout, have a relatively large bud at the apex of the branch, and always grow directly upright.

As is usually the case among synoicous mosses, the antheridia appear first. The first antheridium arises from an immediate segment of the apical cell. Very quickly the apical cell itself becomes involved in the production of other antheridia, so that by the time four or five antheridia have been formed the characteristic structure of the apical cell has been lost, and a broadening region of relatively large irregular cells takes its place. From this meristematic area other antheridia arise.

The antheridium in its development appears to follow the general method already known as characteristic of the Bryales. After a variable number of antheridia (frequently a dozen or more) have been formed, archegonia begin to develop from the cells of the central portion of the meristematic region, which has now grown into a relatively broad, flat-topped receptacle. As a result of this development process, there is a general tendency toward a definite arrangement of the sex organs: a central group of archegonia, and a peripheral group of antheridia. A careful study, however, of the distribution of the

sex organs on a large number of receptacles has shown that there are exceptions to this general tendency, and that the organs are not infrequently intermingled. Thus occasionally an archegonium has been observed among the peripheral antheridia, and young antheridia have often been found growing among the centrally placed cluster of maturing archegonia.

The development of the archegonium has been carefully followed, and in practically all points it conforms with my study (1) of the development of the archegonium of *Catharinea angustata* Brid. A peculiar cytological detail, which was not found in similar organs of *Catharinea*, appears in the archegonia of *Mnium medium*. In the cytoplasm of young egg cells there are always present one or more small bodies, which in general appearance and in staining reaction resemble nucleoli. As the egg cells grow older these bodies increase in size, and, in case there are several present, undoubtedly unite, since but a single body, often exceeding in size the nucleole of the egg nucleus, is generally to be found in the cytoplasm of the egg cell at or near maturity.

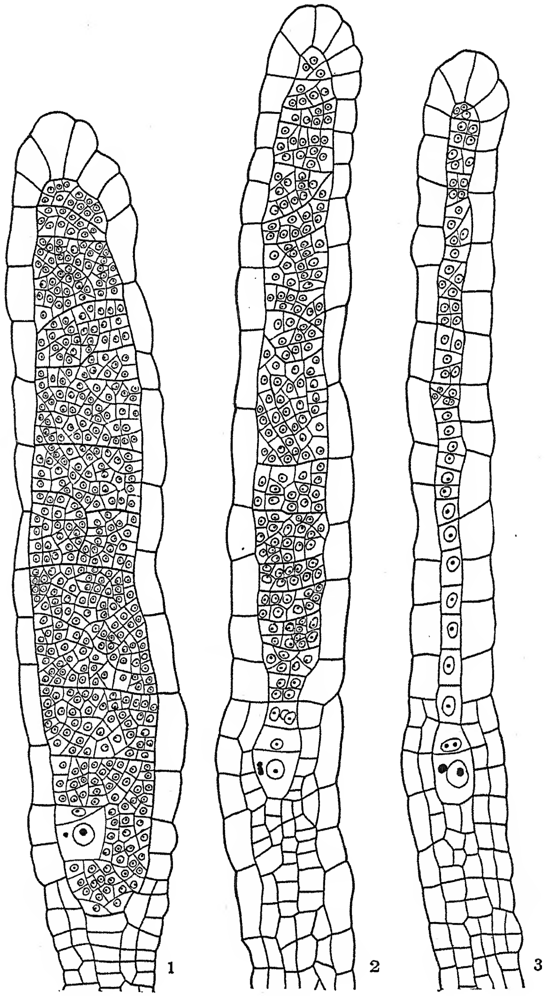
These bodies have been traced back to relatively small globules in the cytoplasm of the ventral cells of the archegonia, and are sometimes quite conspicuous just prior to the division of the ventral cell into ventral canal cell and egg. The cytoplasm of the ventral canal cell rarely shows the presence of these peculiar structures, but they have always been found present in the cytoplasm of the egg. The interesting fact is that these cytoplasmic bodies are conspicuous in what appear to be egg cells in abnormal organs now to be described.

Abnormal sex organs

The great majority of maturing receptacles have shown at least one abnormal organ, and occasionally as many as four have been found on a single receptacle. Most often the abnormalities are located in the transition zone between the two rather well defined groups of sex organs; but they are by no means confined to this zone, as they have also been found among the early formed peripheral band of antheridia and among the later formed central cluster of archegonia. They may thus replace either an antheridium or an archegonium.

The abnormal sex organs have shown a wide range of variation in

the details of their structure. Some of them are very close in structure to normal antheridia, others differ but slightly from normal ar-

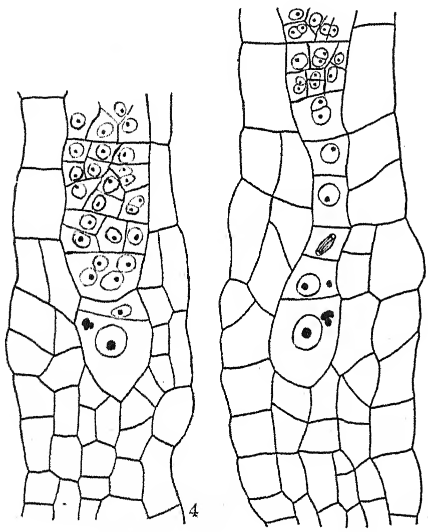


FIGS. 1–3

chegonia. Between these two extremes lie a jumble of forms, many of which are difficult to interpret as to their method of development. It is entirely unnecessary to describe and illustrate all of the varia-

tions that have been found. What appear to be some of the outstanding types have been illustrated, and are briefly described here.

Perhaps one of the most remarkable organs discovered in this investigation is that shown in fig. 1. It is apparently a typical antheridium which is approaching maturity, but at one edge in its lower part are two cells that seem to be, respectively, a ventral canal cell and an egg. Even the cytoplasmic body, which regularly accompanies typical maturing egg cells, is to be found in the cytoplasm of this probable egg cell.



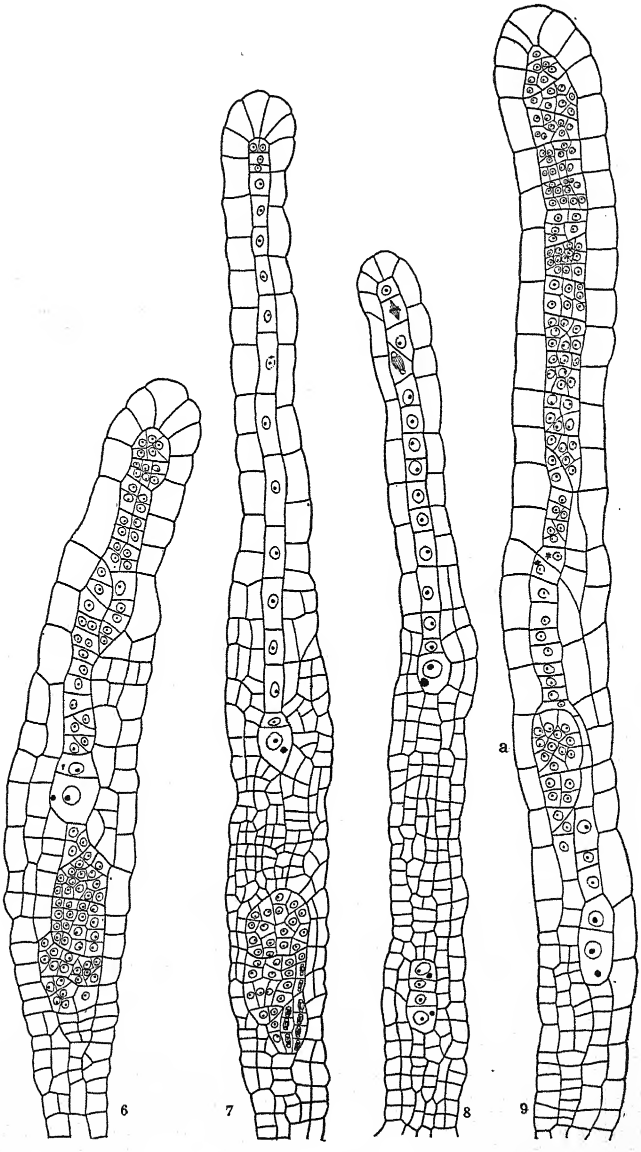
FIGS. 4, 5

Fig. 2 illustrates a type of structure which was found a number of times. The lower portion of the organ is characteristically that of the stalk and venter of an archegonium, and within the venter is a typical ventral canal cell and an egg; but above these two cells lies a structure which, except for its slenderness, is wholly antheridial in nature. In one example of this type (fig. 4) an antheridial-like group of cells is in immediate contact with the ventral canal cell, but in another case (fig. 5) several neck canal cells intervene between the ventral canal cell and the antheridial-like group of cells.

Fig. 3 is characteristic of organs that also were not infrequent in the material studied. Here without question is an archegonium typical in all respects, except that the upper part of the canal row is multiple, a condition which might be described as illustrating an antheridial tendency.

The abnormalities which have been described thus far constitute a very close and remarkable series of organs which bridge the gap between normal archegonia and normal antheridia.

Fig. 6 is rather typical of a peculiar organ that was found several times. There is an apparent venter which incloses a ventral canal



Figs. 6–9

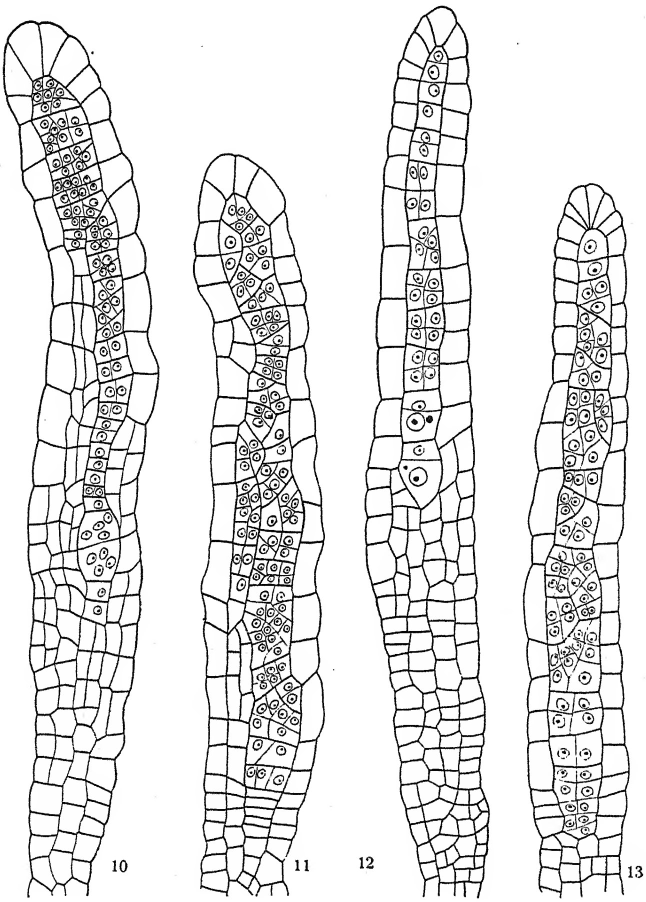
cell and an egg cell. The nucleus of the ventral canal cell, however, is somewhat larger than is generally the case, and a cytoplasmic body is present. It is probable that this ventral canal cell is a potential gamete. Above and below the venter are antheridial-like cells, the basal group particularly resembling the cells of an antheridium. Fig. 7 may possibly be regarded as belonging to the same general category as fig. 6. In any event it presents the anomaly of a well defined antheridial-like mass of cells imbedded in the elongated stalk of a typical archegonium. Two examples of this structure were found in the material studied. A very similar organ is illustrated (fig. 47) by HOLFERTY (3).

Fig. 8 is of interest as showing a gamete-forming tendency of the opposite sex in a structure similar to fig. 7. Imbedded in the lower part of the much elongated stalk of an archegonium is a double venter, the upper ventral canal cell and egg lying in an inverted position. A cytoplasmic body accompanies each probable egg cell. No indications could be found of any cells that might be interpreted as a canal row connecting the double venter with the outside. Two other structures practically identical with the one described and illustrated were found in the material.

Fig. 9 presents a curious mixture of characters. The upper portion is decidedly antheridial in nature, but merges below into an evident row of nine neck canal cells. The outline of what probably began as a venter may be noted at *a*, but no potential egg cell is present, its place being occupied by a number of small antheridial-like cells. Near the base of the organ is an evident venter containing probably two potential gametes. Fig. 10 repeats many of the details found in fig. 9. There is the same upper portion, antheridial in nature, which merges below into a row of neck canal cells. The venter is better defined than in fig. 9, but differs in that each cell within the venter of fig. 10 contains four small nuclei without walls between them.

In all of the organs previously described there has been present at least one well defined egg cell. In fig. 10 there is no indication of any such cell. Each of the eight nuclei within the venter might be interpreted as a potential egg nucleus, but the small size of each is a weighty objection to such a suggestion. With fig. 10 we pass to organs of a type characterized by the absence of any well defined prob-

able egg cell. Such organs were of frequent occurrence in the material studied, and were often found between the peripheral band of antheridia and the central cluster of archegonia. Figs. 11 and 13



FIGS. 10–13

illustrate some of the variations of detail that may be observed in such organs.

Development of abnormal organs

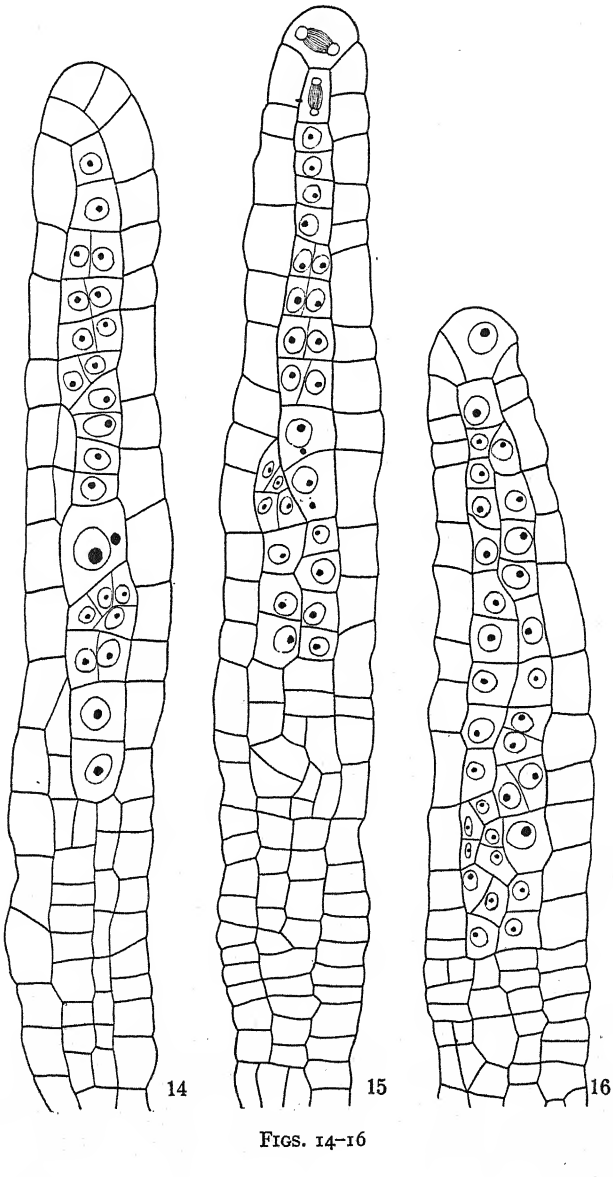
Having found certain types of abnormal organs, an effort has next been made to discover the manner in which these various types

have developed. Some of them clearly tell their own story. Thus there can be little doubt that the organ illustrated by fig. 1 has developed after the manner of a typical antheridium, and that the ventral canal cell and egg have been derived from one of the early segments. Likewise, the type illustrated by fig. 3 shows that it has developed after the method of a typical archegonium, except that the canal cells in the upper part of the neck have divided and redivided an unusual number of times. This unusual number of neck canal cells should undoubtedly be regarded as an antheridial character appearing in an archegonium. Not only do the cells in the canal row resemble in general appearance those of an antheridium, but the nuclei of a number of adjacent neck canal cells have been found to divide simultaneously, which is one of the characteristics of a developing antheridium. These facts now make clear the meaning of the multiple neck canal cells and their simultaneous division observed (1) in some of the maturing archegonia of *Catharinea angustata* Brid.

While the interpretation of the developmental processes of figs. 1 and 3 is reasonably certain and clear, it is quite otherwise with such figures as 2, 6, 9, 10, and 11. For example, in fig. 2 the general manner of development may have been that of an archegonium in which there has been a greater growth and multiplication of the neck canal cells than is shown in fig. 3. On the other hand, the apical cell of the organ may have begun its activity as of the archegonial type, and later may have changed its method of segmentation to the antheridial type, thus explaining some of the irregular divisions in the upper part of the organ. In the hope of throwing some light on this problem of development, a search has been made for early stages of these abnormal organs. Figs. 14, 15, and 16 represent some types of young organs that could be recognized with certainty. Fig. 14 is clearly a young archegonium undergoing modification. Earlier stages of this type could not be recognized with certainty, but are probably represented by certain young archegonia which have unusually broad canal cells.

Fig. 16 is a type quite different from that illustrated by fig. 14. The short stalk, and particularly the breadth and irregular arrangement of most of the potential gamete-producing cells, indicate a modified antheridium. It will be observed, however, that the apical cell

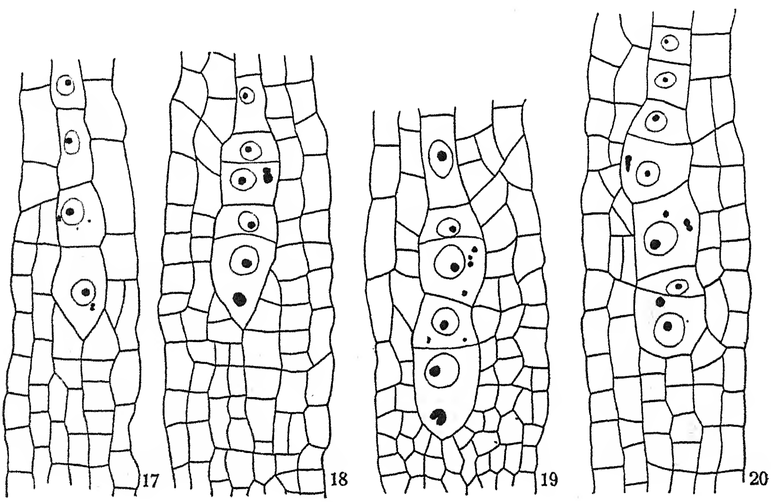
and the segment immediately beneath it are wholly archegonial in structure. It therefore seems probable that this organ began its de-



FIGS. 14–16

velopment by an apical cell of the antheridial type, and that this apical cell has only recently undergone change to the archegonial type. Fig. 13 belongs in the same category as fig. 16, in that it is undoubtedly a modified antheridium. Organs similar to fig. 13 were numerous in the material studied, and are probably represented at an early stage by what seemed to be unusually slender young antheridia.

Fig. 15 seems to belong to the same general type as fig. 16, except that the conversion of the apical cell into the archegonial type occurred at an earlier time in the developmental process.



FIGS. 17–20

The material studied contained a number of examples of double and even triple venters. The origin of a double venter is evident from an examination of fig. 17. In this young archegonium the basal neck canal cell has grown almost as large as the ventral cell. The characteristic cytoplasmic bodies are present in both cells. Fig. 18 is clearly a later stage of such a type of development. Figs. 19 and 20 seem to indicate a method by which triple venters may arise. The ventral canal cell of the lower venter of fig. 19 shows evident tendencies toward the formation of a gamete, as indicated by the size of the cell, of its nucleus, and the presence of cytoplasmic bodies. Fig. 20 probably represents a later stage of development of such a structure as is indicated by fig. 19.

Summary

1. Abnormal sex organs are apparently regularly formed by *Mnium medium.*

2. An abnormal organ may replace either an antheridium or an archegonium, but is most often found on the border between the peripheral ring of antheridia and the central group of archegonia.

3. There are wide variations in the details of structure of the abnormal organs.

4. Many of the organs have developed as modified archegonia; some as modified antheridia; and a few give indications that the apical cell has functioned for a time like that of an antheridium, and later has changed to the archegonial type.

5. A remarkable series of organs linking together antheridia and archegonia have been found.

6. The facts herein presented add further evidence to the hypothesis that the sex organs of the bryophytes are homologous structures.

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BRIEFER ARTICLES

A WATERING SYSTEM FOR CULTURE VESSELS

(WITH ONE FIGURE)

Many experiments have been carried out to determine the water requirements of plants, or the influence of the soil moisture upon their metabolism. The usual procedure has been to grow plants in soil containing varying amounts of moisture. Several watering systems have been devised and used for maintaining a uniform distribution of the moisture through the soil of the culture vessels. KIESSELBACH'S (4) coil watering device and LIVINGSTON'S (5) porous cup atmometer are perhaps the best known. Several others are described by BRIGGS and SHANTZ (1) in their summary of transpiration experiments. These systems, as well as others, seem to work satisfactorily only under certain conditions. KIBBE (3) found that, in crocks equipped with Livingston porous cup atmometers, the roots of the plants collected on the surface of the porous cups, forming a thick mat. The result was that the plants made a comparatively good growth in the "dry" soil when compared with those in medium moist soil. YUNCKER (8), using another watering system, obtained similar results. These results are quite contrary to observations in the field, where drought checks the growth to a considerable extent. SMITH (7) estimates that most crop plants suffer more from deficient water than from all other unfavorable factors combined.

SHANTZ (6) in a recent paper declares that most transpiration experiments will have to be repeated because the experimenters have not taken into account the behavior of the water in the soil. He also points out the difficulties of watering dry soil and obtaining an even distribution below the "field carrying capacity" of the soil. The problem of watering culture vessels satisfactorily seems still to be an unsolved one.

In a culture vessel the soil is relatively loose, so that water will sink into it more readily than into soil in the field. It is for this reason not quite certain whether SHANTZ'S term "field carrying capacity" of the soil is strictly applicable to soil in culture vessels. The looseness of the soil in a vessel makes watering there easier than in a field. Watering on the surface of the soil gives, within reasonable limits, a rather uniform distribution of moisture throughout the vessel for a short time, provided the water

is applied slowly. However, water applied in this manner will soon cause a hard crust to form from the top layer of the soil, provided it is not extremely light. This crust, being more compact, absorbs more water than the lower layers of the soil, and interferes with the gas exchange of the lower layers, preventing satisfactory results. Some improvement is produced by sinking a small flower pot in the center of the filled vessel and adding the water into this pot, keeping the surface of the soil unwatered; but in vessels where a uniform distribution of a smaller amount of moisture than the optimum for plant growth is required, this system does not give the desired uniformity.

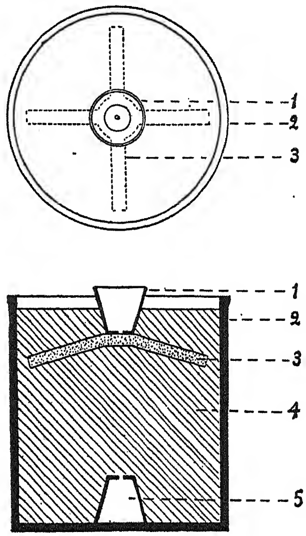

FIG. 1.—Diagram of watering system for uniform distribution in culture vessels: 1, small flower pot for watering; 2, culture crock; 3, sand for spreading water horizontally; 4, soil; 5, small flower pot serving as air space.

In an experiment in which plants were to be grown under three conditions of soil moisture, dry, medium moist, and moist, the writer found the following watering system to give a very uniform distribution of the soil moisture in the dry vessels, the ones which always have caused the most trouble. In the bottom of each vessel, which in this experiment was a one gallon glazed crock, a small flower pot was placed to serve as an air space. The necessity of this pot may be questioned. The crock was then almost filled with air-dry soil, consisting of silt loam with 10 per cent fine sand. The surface of the soil was kept higher in the center of the crock. With a finger, four radiating grooves were made in the surface of the soil and filled with coarse sand, a small flower pot was placed in the center of the cross, and the remainder of the soil added (fig. 1). The radiating arms of sand were covered with about one inch of soil. In vessels larger than those used in this experiment a larger number of radiating arms of sand may be necessary to obtain the desired result. This watering system seemed to work very satisfactorily. Three degrees of soil saturation were maintained, 15, 30, and 45 per cent of the moisture-holding capacity of the soil as determined by HILGARD's (2) method. The plants grown were wild mustard and oats together, for the purpose of obtaining plants with an

anatomical structure modified by the degree of soil moisture. It was found that plants grown in vessels containing dry soil, compared with those grown in the medium moist soil, greatly resembled plants grown under dry and moist conditions in the field. Five dry cultures gave a yield of leaves of wild mustard of 2.9 gm. dry matter, while five cultures from the medium moist series gave 7.8 gm. yield; the leaf areas were respectively 8.83 and 32.73 sq. dm. These results seem to confirm SMITH's statement about the necessity of water for crop growth, and do not agree with the reports of some workers who were able to obtain almost as good growth in "dry" cultures as when water was supplied liberally.

If a watering system in dry soil gives an uneven distribution of the moisture, the roots seem to develop most in the moist region of the soil in the vessel, as was the result in KIBBE's experiments. The development of the roots seems, for that reason, to be a fair indication of the efficiency of the watering system used. Plants growing in dry soil watered by the method here described in no case showed any conspicuous development of the roots in the upper part of the soil; usually the roots were uniformly distributed throughout the whole mass. Thus this system seems to make it possible to provide a uniformly dry soil for growing plants in culture vessels.

An additional feature of this system is that it makes it possible to water the cultures quickly and easily. In vessels equipped with this watering system the water was absorbed in one minute or less, while in vessels without the arms of sand the absorption of the water required several minutes.—ALFRED ASLANDER, *Department of Botany, Cornell University.*

[*Accepted for publication January 26, 1927*]

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NOMENCLATORIAL NOTE ON SMITHIANTHA FULGIDA (ORTG.) CHENEY, COMB. NOV.

A recent study of the Mexican and sometimes cultivated plant of greenhouses known as *Naegelia fulgida* Ortg. shows that it is incorrectly designated by the present name. The genera *Naegelia* and *Smithiantha* of the Gesneriaceae (Metachlamydeae) have been confused, due to the improper generic use of the term *Naegelia*. Investigation reveals that *Naegelia* was first employed by Rabenhorst in his *Kryptogam–Flora* (85, 1844), for a genus of Saprolegniaceae. This original application of the generic name *Naegelia* is still credited as valid by the mycologists, and is listed in Oudeman's *Enumeratio Fungorum* (1:111. 1919).

Apparently unaware of the chronology of publication or of the actual existence of the fungal genus of Rabenhorst, one finds that several authors have described genera in various groups of plants under the term *Naegelia*. In Zoll. and Mor. *Syst. Verz. Zoll.* (1845–46) *Naegelia* is applied to a genus of Rhamnaceae which is now synonymous with *Lupulus* O. Ktze. of the Rhamnaceae. Lindley used the same name (*Naegelia*) in 1845 for a genus of Rosaceae; see also Lindley's *Veg. Kingdom* 560, 1847. Lindley's genus is now equivalent to *Malacomeles* Dcne. The term *Naegelia* of Regel in *Flora* 31: 249. 1848, for a genus of Gesneriaceae was absorbed in 1891 by the *Smithiantha* of O. Kuntze in his Rev. Gen. Pl. II. 977, 1891.

The species *fulgida* was described under *Naegelia* by Ortgies, in Regel, *Gartenfl.* 97, 1867. In consideration of the change in generic terminology making the combination *Naegelia fulgida* Ortg. impossible, and the fact that an examination of herbarium specimens substantiates the species description by Ortgies, the combination should be **Smithiantha fulgida** (Ortg.) Cheney, comb. nov.—Ralph H. Cheney, *Washington Square College, New York University, New York.*

Decapitalization of specific names

Commencing with this issue, the Botanical Gazette will abandon the use of capitals for all specific names, thus meeting the wishes of an increasing number of contributors, and following the practice of Biological Abstracts and most biological journals.

CURRENT LITERATURE

BOOK REVIEWS

Plant ecology

Teachers of plant ecology will welcome the appearance of McDougall's book[1] presenting an elementary treatise of this subject, and especially designed as a textbook for use in beginning classes. The work is the outgrowth of the author's experience, extending through several years, in teaching beginning classes in plant ecology at the University of Illinois, and the chapters of the book are based on the lectures prepared for these classes.

The introductory chapter defines the subject and points out the importance of such a study of plants. The next three chapters deal with the environmental relationships of the three fundamental vegetative systems of the plant, root, stem, and leaf. In the chapter on stems considerable space is devoted to a description of the various tissues of the stem. This account is necessarily very brief, and so condensed that a student without any previous knowledge of stem structure could scarcely be expected to form any adequate idea of these component tissues from the facts presented. It is to be assumed that a student of plant ecology would probably have had an introductory course in botany, and in that case this portion of the chapter would represent merely a review of the subject. It may have been so intended, but in the reviewer's judgment this material should have been replaced by a brief discussion of the structural modifications induced in these tissues by various environmental factors.

Chapters V to IX develop a very complete and thorough analytical presentation of the subject of symbiosis. Social and nutritive types of symbiosis are described for each of two larger classes of symbiotic phenomena, disjunctive and conjunctive symbiosis. Much confusion occurs in the writings of various authors dealing with this subject, and the clear elucidation of the facts recorded in these chapters should enable the student to steer clear of the uncertainty that so often develops when an attempt is made to understand the classification of symbiotic phenomena.

Chapters X to XIV deal with the five important physical factors of the plant's environment, light, temperature, air, soil, and water. The facts are tersely presented and the discussion is very much restricted, but the scope of the presentation is such that all the salient features necessary for an elementary course are included, and by making use of more comprehensive reference works

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[1] McDougall, W. B., Plant ecology. pp. vi+326. Philadelphia: Lea & Febiger. 1927.

the teacher can extend the discussion as much as may be desired. Chapter XV discusses the characteristics of plant forms that have developed in response to a certain uniformity of environment, and that are distinctive of plants growing in those habitats where such a degree of uniformity obtains. This chapter also includes a brief discussion of the life forms of RAUNKIAER. Chapters XVI to XX deal with synecology. The idea of plant succession is very ably presented, and the terminology used in the classification of plant groupings is clearly defined. The author is at his best in the development of this portion of the text, and these chapters reflect something of his genuine pleasure in this phase of ecological study. Chapter XXI presents the subject of phenology, with a brief discussion of certain seasonal aspects of vegetation, and chapter XXII gives an account of the distribution of some of the major plant communities, with the idea of merely introducing the subject of plant geography. Chapter XXIII is concerned with the subject of applied ecology. Keeping in mind the work of BRIGGS and SHANTZ, COWLES, SAMPSON, WEAVER, KIESSELBACH, and many others who have demonstrated the importance of ecology in its application to the solution of practical problems in agriculture, the teacher will be glad to note in the final chapter of the book the emphasis that McDOUGALL has placed on this phase of ecological work. There is also an appendix of ten pages which gives a large number of general suggestions concerning laboratory and field work.

The volume is handsomely bound, and reveals a high grade of excellent workmanship in every detail. The text is printed in clear, bold type, and is surprisingly free from typographical errors. There is an abundance of good illustrations, well chosen to clarify and enliven the descriptions of the text.

As the first elementary American textbook in plant ecology, McDOUGALL's book will doubtless receive its full share of criticism. There are some who will find fault with the organization of the material, but there is internal evidence in the book that such criticism already exists in the mind of the author himself. No subject has ever yet been presented in an ideal textbook, and certainly no such book may yet be anticipated in a scientific field so young as that of plant ecology. However, all must agree that McDOUGALL has produced an excellent pioneer work, and it is to be hoped that his venture may encourage a deeper and more extended interest in the adequate presentation of the subject of plant ecology.—P. D. STRAUSBAUGH.

Enzymes

Biologists have long felt the need of a recent, authoritative book on enzymes. There are recent works which deal with the nature of enzymes and their action, but books which treat enzymes in a broad general way have been notably lacking. One has had to rely on chapters in books dealing with plant and animal chemistry.

In their recent book WAKSMAN and DAVISON[2] have done much to supply

[2] WAKSMAN, S. A., and DAVISON, W. C., Enzymes, properties, distribution, methods and distribution. pp. xii+364. Baltimore: Williams and Wilkins Co. 1926.

this need. The volume is divided into four sections. The first considers the properties of enzymes. The introductory chapter gives a clear statement of the historical development of our conception of enzymes, defines them, and gives their criteria and a table of classification. Chapter II of this section considers the rôle of enzymes and their secretion, and treats proenzymes, coenzymes, antienzymes, and the specificity of enzymes. Chapter III takes up the chemistry of enzymes and enzyme reactions. Our lack of knowledge of the actual chemical nature of enzymes is reflected in the small amount of space devoted to this subject. Chapter IV considers the factors that affect enzyme activity.

The second part of the book treats the distribution of plant and animal enzymes. The relatively large amount of work that has been done on animal enzymes is indicated by the fact that over half of the sixty pages of this section is devoted to human and animal enzymes, the other pages treating plant enzymes and enzymes of the microorganisms. Part III contains 125 pages, devoted primarily to a consideration of the methods of preparation and study of the different enzymes, but containing much material on the occurrence and mode of action of the enzymes. The different groups of enzymes as given in the table of classification are treated here. An interesting and useful section is the last one, in which is given a consideration of the practical applications of enzymes. One is impressed by the many uses to which enzymes are put in the industries and in various practical lines.

The volume is more a reference book than a textbook. The successful attempt has been made to give a concise summary and evaluation of the vast, often contradictory literature on enzymes, and thus lay the basis for future work on this subject. Important features of the book in this connection are the extensive bibliography, over 1350 references, and the large amount of space devoted to a consideration of the methods of preparation and study of enzymes. The book should be of great value to the teacher, the investigator, and the worker in the various applied fields where enzymes are used.—S. V. EATON.

Oeuvres de Pasteur

In assembling into one authoritative text the scattered papers of PASTEUR, VALLERY-RADOT[3] has performed a great service to biology in all its branches, to chemistry, and to the scholarly world generally. It is very gratifying to have the new development in physiology and bacteriology redacted and authenticated within the lifetime of men who knew the great teacher and are themselves not yet old. VALLERY-RADOT has applied himself to his task with an industry and thoroughness worthy of its magnitude, and the publishers have not lagged behind him in the typographical and mechanical work. The type has that beautiful face that only Continental designers seem able to achieve, and the reproductions of the original illustrations have been excellently and in some instances strikingly arranged. The four volumes thus far received in this country cover

[3] VALLERY-RADOT, P., Oeuvres de Pasteur. vols. 7. Paris: Masson et Cie.

PASTEUR's earlier chemical researches, his explosion of the spontaneous generation theory, studies on wine and vinegar, and the classical work on the diseases of silkworms. The three yet to come will cover his studies on beer, his treatises on virulent diseases and their prophylaxis, especially rabies, and a final volume of miscellanies. By the simple fact of its existence, this edition imperatively demands a place in every scientific library. The price is so low, only about $16 at current exchange rates for the four volumes thus far issued, that even small institutions can afford to purchase it.—F. THONE.

Plant geography

There has long been a need for a general volume on plant geography, less technical than that of SCHIMPER and DRUDE, and designed primarily for the amateur who desires an acquaintance with the vegetation of the different countries of the world. It is not unfitting that such a book has come from the hand of a plant morphologist, who expressly disclaims being a specialist in the field of plant geography. CAMPBELL[4] has issued a volume which should be in the hands of all intelligent world travelers, for one without technical knowledge will have no difficulty in following through the volume. At the same time the student of botany desiring to get a picture of the large features of world vegetation will find this book very helpful. The author is himself a great world traveler who has visited many countries in search of morphological material, and he has also published various important phytogeographic papers on Hawaii and other lands.

The introduction deals with the succession of plants in geologic time, then follows a chapter on climatic zones. Seven chapters deal with the vegetation of the different lands as follows: Eurasia and North Africa, Atlantic and Central United States and Canada, the Rocky Mountains and Pacific slope, Africa and Continental Asia, Malaya and Polynesia, the Neotropical Regions, and the South Temperate Zone. The volume is profusely and well illustrated by photographs taken by the author and others.—H. C. COWLES.

Soil microbiology

Once in a while a book appears that one knows is destined to become a classic in its particular field, and to pass through edition after edition. Such a book is WAKSMAN's[5] new work, an encyclopedic summation of our knowledge thus far in the literature of the little things that live in the soil unseen and for the most part unthought of, yet vastly influencing our lives for good or ill.

Waksman chooses his text well, from PASTEUR: " . . *le rôle des infiniments*

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[4] CAMPBELL, D. H., An outline of plant geography. 8vo. pp. ix+392. *pls. 52. figs. 101.* New York: Macmillan Co. 1926.

[5] WAKSMAN, SELMAN A., Principles of soil microbiology. 8vo. pp. xxviii+897. *pls. XIX. figs. 77.* Baltimore: Williams & Wilkins. 1927. $10.00.

petits m'apparaissait infiniment grand . . ," and the thoroughness with which he undertakes his task measures well with his appreciation of its importance. Bacteria, protozoa, myxomycetes, fungi, algae, worms, all the swarming invisible life of the earth: he neglects none of them. Nor does he neglect an avenue of approach that promises to lead into a region whose exploitation will be profitable: taxonomy, physiology, ecology, physics, chemistry, each comes in for its share of attention, with special chapters on oxygen, nitrogen, and sulphur relations, and whole series of chapters on the interactions between the living and non-living organic masses in the soil. He pins his generalizations down with tables of quantitative data, 94 of them. He has impounded and digested a huge literature; his classified list of useful reference books fills 8 pages, his index of authors 21, and his separate citations reach the impressive total of 2543. In quantity the book is impressive; in quality it is excellent.—F. THONE.

Palladin's Plant physiology

The PALLADIN-LIVINGSTON[6] textbook of plant physiology has appeared in its third English edition. Only a few changes have been made from the second edition. The list of books for reference preceding the table of contents has been extended considerably, with a gain in value to the general student, as many of the references added are of the newer publications. Some of the footnotes have been revised to include recent publications also, and an account of the ASKENASY experiment has been inserted on pages 147–150.

One small error has persisted through all three editions, and attention is called to it, in the interests of students who may try repeating the demonstration of oxygen liberation during photosynthesis by use of the SCHÜTZENBERGER reagent. The directions are given on page 5, but sodium sulphite, Na_2SO_3, cannot be used for this purpose. The original formula calls for sodium hyposulphite. Unfortunately sodium thiosulphate, $Na_2S_2O_3$, has frequently been called sodium hyposulphite, and goes as "hypo" in photography. The proper chemical to use is the true sodium hyposulphite, $Na_2S_2O_4$.

The book has been very useful since its first appearance, and will no doubt continue to be used by students of plant physiology until something better has been produced in the way of a textbook.—C. A. SHULL.

NOTES FOR STUDENTS

Boron, a plant nutrient.—Since the first discovery of boron in plants 70 years ago, the widespread occurrence of boron in fruits, leaves, and other parts of plants throughout the plant kingdom has been abundantly demonstrated. The toxic action of excessive quantities of boric acid and borax has also been observed by many investigators. Occasional suggestions also were made of stimu-

[6] PALLADIN, V. I., Plant physiology, translated by LIVINGSTON, B. E., 3d ed. 8vo. pp. xxxvi+360. Philadelphia: Blakiston. 1926.

lating action of very dilute solutions of boron compounds; but in recent years evidence of a more important relationship of boron to plant life and plant growth has been obtained.

Since the work of AGULHON, 17 years ago, experiments have been in progress at the Rothamsted Experiment Station which demonstrate the importance of boron to some of the legumes. The work was begun with *Vicia faba*, and Miss WARINGTON'S[7] results proved conclusively that boron was needed by the broad bean. Plants grown in nutrient solutions spectroscopically free from boron died in a characteristic manner, the growing points being the parts mainly affected. The amount of boron needed was so small that mere traces were sufficient to promote normal growth. The root systems without boron were short and stumpy, whereas they were long and well branched if traces of boron were present.

The abnormal behavior of this plant in the absence of boron is irrespective of the pH of the nutrient solution, of the aeration, of the nature of the substratum, or of the presence or absence of nodules on the roots. The relation of boron to the nodule development on *V. faba* was investigated by BRENCHLEY and THORNTON,[8] who claim that boron is indispensable to the legume bacteria which use it in some way in connection with the nitrogen fixation process, the synthesis of organic nitrogen compounds from atmospheric nitrogen.

The various anatomical changes in the structure of *V. faba* by the absence of boron were reported by Miss WARINGTON,[9] who found that both stem and root structures are modified by lack of boron. Cambium cells become hypertrophied, followed by degeneration, or they may disintegrate without hypertrophy. The phloem and ground parenchyma also show some disintegration, and the xylem is poorly developed. Whether these effects are due to the absence of some direct action of boron, or to indirect consequences, is not yet certain. It is suggested that the various changes are correlated with the meristematic activity of the tips of stems and roots.

Although any boron-containing compound, even the insoluble borates, supply the plants sufficiently, no other element can be substituted for boron in this connection. BRENCHLEY and WARINGTON[10] have tested 52 other elements,

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[7] WARINGTON, KATHERINE, The effect of boric acid and borax on the broad bean and certain other plants. Ann. Botany **37**:629–672. 1923.

[8] BRENCHLEY, W. E., and THORNTON, H. G., The relation between the development, structure, and functioning of the nodules on *Vicia faba*, as influenced by the presence or absence of boron in the nutrient medium. Proc. Roy. Soc. London B. **98**: 373–398. 1925.

[9] WARINGTON, KATHERINE, The changes induced in the anatomical structure of *Vicia faba* by the absence of boron from the nutrient solution. Ann. Botany **40**: 27–42. 1926.

[10] BRENCHLEY, W. E., and WARINGTON, KATHERINE, The rôle of boron in the growth of plants. Ann. Botany **41**: 167–187. 1927.

with special emphasis on manganese without finding a successful substitute. They have extended their observations to include several leguminous plants, melon, cereals, and candytuft. The legumes and melon require boron, but cereals and candytuft complete their development without it. In this their results do not agree entirely with those of SOMMER and LIPMAN,[11] who found that boron was essential to the complete development of barley, sunflower, cotton, buckwheat, castor bean, flax, and mustard. BRENCHLEY and WARINGTON think the distinction between those plants that need boron and those that do not may not necessarily be qualitative, but rather quantitative. Some plants may have enough boron in their seeds to supply the amount needed for complete development.

Renewal of the nutrient solution by means of drip cultures in which renewal was complete every 24 hours retarded the appearance of boron starvation for from 3 to 6 weeks, but did not prevent ultimate death of the plants. In their most recent paper BRENCHLEY and WARINGTON express the belief that there is an association between boron and the absorption and utilization of calcium. The boron is not considered an ordinary catalyst, but seems to be absorbed and combined, or removed from action in some way, so that a constant supply seems to be needed.

The amount of boron needed by plants is so small, about one-half part per million, that it can never be a problem in nature. All soils naturally contain enough boron that to demonstrate the need of plants for this element requires unusual precautions in the way of insoluble glass vessels, recrystallized salts, and protection from dust particles. Some of the other elements now considered nonessential for plant growth will no doubt be found necessary when we actually provide the plant with an environment entirely free of the elements in question. Plants have probably never been grown in the complete absence of silicon, for instance, nor of chlorine. Until they are, we cannot decide whether these elements are necessary or not to the normal growth of plants. Boron has now been shown to be indispensable in minute amounts, for the proper development of many of the higher plants.—C. A. SHULL.

North American Flora.—The fourth part of volume 34 contains the presentation of 3 tribes of Carduaceae by AXEL RYDBERG. The Liabeae include 5 genera, 3 of which are new, namely *Sinclairiopsis*, *Megaliabum*, and *Liabellum*. The largest genus is *Sinclairia*, with 18 species. Nearly all of these forms have been segregated from *Liabum*. The tribe Neurolaeneae contains 2 genera, with 15 species, 5 of which are new. The tribe Senecioneae is presented with 15 of its 27 genera. Much the largest genus is *Arnica*, with 107 species, 31 of which are new. The 14 remaining genera include 33 species, 5 of which are new. Among them are the following new genera: *Raillardiopsis*, *Pseudobartlettia*, and *Psathyrotopsis*.—J. M. C.

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[11] SOMMER, A. L., and LIPMAN, C. B., Evidence on the indispensable nature of zinc and boron for higher green plants. Plant Physiol. 1:231–249. 1926.

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A WORKING HYPOTHESIS ON THE ORIGIN OF RUSTS, WITH SPECIAL REFERENCE TO THE PHENOMENON OF HETEROECISM

CLAYTON ROBERTS ORTON[1]

Heteroecism was discovered by SCHÖLER in 1818, and was finally established on an adequate experimental basis in 1865, by DE BARY and ORSTED independently. Its origin remains a mystery, however, in spite of the numerous efforts to explain the phenomenon, chiefly on the grounds of the comparative development of the life cycles of the rusts.

FISCHER (16) considered the ancestors of our present heteroecious rusts to have been autoecious, but able to live on a wide variety of hosts. In such a plurivorous state, a rust may have been capable of developing its entire cycle upon plants representing the host of the present haploid generation, as well as upon those bearing the diploid. Heteroecism was thought by FISCHER to have originated by a progressive specialization, in which some of the rust offspring acquired an adaptation of their aecial stage to one set of hosts, and the telial stage to a different set of hosts. He believed also that the short-cycle forms originated by reduction and specialization from these same plurivorous parental types.

The earlier views of DIETEL (10), which have been adhered to by many students of the rusts, hypothecated the primitive rust as a

[1] Read before the Mycological Section of the Botanical Society of America at the Washington Meeting, December, 1924.

Contribution from the Department of Botany, Pennsylvania State College, no. 59.

short-cycle lepto-form or micro-form, which normally inhabited the aecial host of the present heteroecious species. By extending its vegetative stage to produce aecia and uredinia, it somehow lost its ability to complete this more complex cycle on the one host, and so transferred its sporophytic stage to a different type of host. DIETEL (13) claims to have followed DE BARY'S views on *Chrysomyxa* in regard to the presentation of his early ideas on the origin of heteroecism.

KLEBAHN (25) held that the host-changing species originated from the autoecious fixed types by moving the telial generation to other plants, which he thought was possibly explained by the influence of internal factors, such as mutation. He also considered it possible that the aecial stage was the one transferred to another host in certain cases, and DIETEL (12) also holds this latter opinion, particularly in the case of *Melampsora*. MCALPINE (29) holds essentially the same views as KLEBAHN.

A somewhat different view has been expressed by BARCLAY (4) and GROVE (21), who have proposed that the Aecidiaceae have been derived from *Endophyllum* by amplification and adjustment of the extended sporophyte to a new host.

BLACKMAN (7), on the basis of his discovery of cell fusion in the base of the aecium and nuclear reduction in the basidium, resulting in the establishment of an alternation of generations, presents the probable primitive condition of the rusts from a different angle. He states:

> As the heteroecious forms are confined to those possessing the aecidium, that is, to the more primitive, it seems probable that heteroecism may not be, as generally conceived, a later adaptation, but may actually be the primitive condition in the group. Although we are ignorant of the origin of the group, it is possible to conceive that the sporophyte was first developed in connection with a new terrestrial existence. The autoecious eu-forms would then be the first step in reduction, a purely environmental one; later a morphological reduction of the number of spore-forms would appear to have taken place.

OLIVE (35), in accord with DIETEL'S earlier views, holds the primitive rust to be the short-cycle type, fixed on the present host of the haploid stage. The passing of the extended diploid stage, which he postulates as a later development, to an alternate host, is considered by him to be brought about by the stimulus afforded

by the fusion in the base of the aecium, thus enabling the more vigorous aeciospores to be capable of infecting a new and different host.

More recently DIETEL (13) has reopened the subject, and taken the occasion to revise his views regarding the primitive type. He now concludes that the short-cycle species have arisen generally through reduction, since from their numbers, distribution, and host relationships they can no longer be regarded as primitive. On the other hand, he holds that where a host change was effected, it was brought about generally by the sporophytic stage passing to some other host. In the Melampsoraceae, however, which behave in an opposite manner to the Pucciniaceae, he believes that the original hosts of the oldest forms (like *Uredinopsis*, etc.) were ferns, and that they adopted the Abietineae as new hosts for their aecial stage, when these plants appeared.

ARTHUR (2) believes that the fern rusts show morphological evidences of being most primitive, and that the conditions of heteroecism and pleomorphy were primitive also.

The excuse for reopening the subject lies in the fact that a considerable body of information is now available which either has never been presented in this connection, or has not been given adequate attention. A discussion of the views held by the previous writers will be reserved for the summary.

Significance of alternation of generations

The alternation of generations persists in the rusts despite the loss of sex organs, indicating certainly that it is an inherent character, and it follows logically that these generations would be separated in the case of heteroecious rusts.

Since the discovery by BLACKMAN of the nuclear migrations and cell fusions at the base of the aecium, and that of SAPPIN-TROUFFY showing that chromosome reduction in the basidium precedes the initiation of the gametophyte, no adequate attempt has been made to point out the significance of these facts in the origin of the rusts and their phylogeny. Compelled as we are to assume that the Uredineae originated from independent plants, one of the most significant features of the group is the fact that a definite alternation of

generations is present throughout, even in the short-cycle forms in which the diploid phase is much reduced. If there is no significance in the alternation of generations conforming to the heteroecious habit, then there would appear to be no reason why the separation in the life cycle of heteroecious rusts should not take place between the uredinial and telial stages, with uredinia borne on the host for pycnia and aecia. This is what might be expected to occur if the contentions of CHRISTMAN (8), MCALPINE (29), and OLIVE (35) are correct, but as yet no rust has been found where such a separation occurs.

The failure of the two nuclei to fuse in the basal cell may possibly be explained on the ground that in the rusts the sex apparatus is lost, and that cell fusion, as DODGE suggests, is secondary like that in the red algae, in which the auxiliary cells take part. That the paired nuclei are not completely antithetic is evidenced by their eventual fusion in the teliospore, and a number of cases are now known where nuclear fusion takes place in the aeciospores. That such an apparent abnormality should occur in a parasitic group of plants and possibly become a permanent condition is not surprising. The fact that certain rusts, such as *Endophyllum uninucleatum* (MOREAU 33), and the uninucleated forms of *Tranzschelia punctata*, *Ochropsora sorbi* (KURSANOV 26, 27), *Uromyces rudbeckiae* (OLIVE 35), *Endophyllum centrantheri-rubri* (POIRAULT 38, 39), *Kunkelia nitens* (DODGE 15), and other rusts are known to complete their life cycle in the haploid condition only, may be further evidence that the parasitic habit may lead to extreme results even in morphological degeneration.

Conservation or fixity of organs

It appears logical to expect the most persistent organ in a rust to be homologous with the most characteristic organ of its independent ancestor. Such a relation appears between the teliospore and the tetrasporangium of certain red algae. The doctrine of the conservation of organs holds an important place in phylogeny in the theories of students of the higher plants. Comparative studies of early geologic forms and of related existing ones have shown that certain organs are more likely to persist unchanged than others. Application of this doctrine to the fungi has been restricted for several reasons.

Unfortunately, we are not aware at this time of the presence of any considerable number of fossil rusts to guide us on these points. The few uncertain cases cited by MESCHENELLI (32), however, are aecidioid (Aecidites) and telioid (Puccinites) types, indicating that aecia and telia are the more fixed, and probably the most primitive spore stages of our present rusts.

The teliospore with its basidium appears to represent the most conservative persistent structure of the rusts. I have come to this conclusion after carefully considering the matter from the standpoint of the spore structure most universally present in the group. Strictly speaking, the 4-celled basidium is the most outstanding stage in the development of the rusts, and we can logically consider this as their most conservative structure. It would be logical as well to expect the teliospore as the most conservative organ, since it is a tetraspore mother cell. Reasoning from this standpoint, we can see at once the relationship between the teliospore and the tetrasporangium, since both produce four spores as the result of maturation divisions.

Effect of acquisition of parasitic habit

The acquisition of a parasitic mode of life on the part of a plant, formerly independent, produces certain very definite changes. The most prominent is the loss of chlorophyll, and this feature is well represented in numerous algal forms like *Harveyella*, which are parasitic on other algae and show various gradations in loss of chromatophores and chlorophyll formation, apparently in proportion to the degree of dependence attained. SETCHELL and others have noted a number of these parasitic red algae. Other definite changes are embodied in the development of the thallus. This is invariably reduced in size and often in structural differentiation. Such changes certainly point to degenerative processes which may be expected to affect the reproductive parts as well. While the evidence as to how parasitism has with certainty modified reproductive structures in the rusts is not substantiated, unless we admit *Kunkelia nitens* to this category, we can scarcely avoid arriving at the logical conclusion that reductions rather than amplifications are to be expected in any group of obligate parasites. It is difficult to conceive how such a

mode of life, which plainly results in degeneration of vegetative parts, could bring about the opposite effects on reproductive parts, especially such changes as would be involved in the development of a long-cycle from a short-cycle form.

Length of cycle as indicating trend of phylogeny

The evidence gleaned from a study of the relative numerical importance of the long and short cycle in the rusts, their geographical distribution, their correlations, and their climatic adaptations, indicates clearly that the phylogenetic trend is toward the short-cycle form, in both heteroecious and autoecious species. We conclude, therefore, that long-cycle rusts are the more primitive.

The bearing which the length of cycle of specific rusts holds to the phylogeny of the group has been given considerable attention by FISCHER, DIETEL, MAGNUS, KLEBAHN, and others. Without entering into a discussion of the several views held, it may be stated that the short-cycle rusts have generally been accepted as the more primitive, and have in various ways given rise to the long-cycle forms by amplification of the sporophyte. BLACKMAN (7), KURSANOV (27), and those agreeing with ARTHUR, have taken the stand from the evidence at hand, that the trend of evolution in this group has been in the opposite direction; that is, that the long-cycle forms are the more primitive, and that the short cycles have originated later through the reduction of the sporophyte. DIETEL, who earlier held the former view, has more recently (13) reviewed the whole matter, and come to the conclusion that the latter method is the more likely one. The conclusions of KURSANOV from his independent researches add much weight to this conclusion. The data bearing on this point have been gathered from several sources, and approach the problem from four angles.

1. RELATIVE NUMERICAL IMPORTANCE OF SHORT-CYCLE FORMS.—There are recognized in the North American flora approximately 1200 species of rusts. Of these about 200 are short-cycle lepto-forms or micro-forms. If it is to be considered that the short-cycle rusts are the more primitive, then we are forced to acknowledge that a large majority of our rusts have already undergone a pronounced change by lengthening the sporophytic stage, and if this is the case

we should also expect to find evidences of this lengthening process among our representative species. No such cases are known at present; on the contrary, we feel confident that the opposite condition is now taking place, as is evidenced through the studies of KUNKEL and DODGE upon the two forms of the orange rust of brambles (*Gymnoconia interstitialis* and *Kunkelia nitens*). In this case there can be no doubt that the short-cycle *Kunkelia* has been derived by reduction from the long-cycle *Gymnoconia*. Furthermore, the short-cycle form is apparently undergoing still further changes, as indicated by the recent studies of DODGE (15) pointing out a distinct form which he has discovered. There are other rusts which point strongly toward a similar process of reduction, but further proof should be presented. It then appears that the most logical theory, which is upheld by at least one known case, is that the short-cycle rusts are being evolved from the long-cycle forms, which fits best with the data on the relative numerical importance of the two cycles. In this connection it is well to point out, as KERN (24) has done, that in the Campanulales, which are acceded to be our most recently evolved flowering plants, the proportion of long-cycle to short-cycle species of *Puccinia* is one to two. This would indicate that the short-cycle forms are in the ascendancy in this order of host plants.

It may be well to point out also, that of approximately 200 short-cycle species known in America, 149 belong to *Micropuccinia*, as delimited by ARTHUR. It must be significant that such a large proportion is to be found in a genus which most uredinologists regard as the most recent in phylogeny, as exemplified by the type of teliospore and structure of its sorus.

2. GEOGRAPHICAL DISTRIBUTION OF SHORT AND LONG-CYCLE SPECIES.—DIETEL (13) has presented the most complete data on the geographical distribution of the short and long-cycle rusts, and has shown that almost invariably the short-cycle forms, which are correlated with long-cycle species, are more restricted in their distribution. So far as he knows, only one short-cycle species, *Uromyces phyteumatum*, occupies a wider geographical distribution than its corresponding long-cycle form, *Uromyces caricis-sempervirentis*. Of twenty-three short-cycle forms cited by ARTHUR and JACKSON (1) as definitely correlated with eu-heteroecious species, fourteen

have a very restricted distribution; six approach their corresponding heteroecious species in distribution; two are practically coextensive; and one, *Puccinia holboellii*, has a wider distribution than its correlated *P. monoica*. These data agree in the main with those of DIETEL. It might be argued that the long-cycle forms are better adapted to extend their distribution through the possession of the repeating urediniospores, but that such a conclusion is unwarranted is brought out in the next point.

3. CORRELATIONS BETWEEN SHORT AND LONG-CYCLE RUSTS.—When DIETEL (9), FISCHER (16), and TRANZSCHEL (41) established the connections between short and long-cycle rusts, the former occurring on the aecial hosts of the latter, they disclosed a condition of the greatest import in the phylogeny of the group. A considerable list of such species has been compiled by DIETEL (13) and ARTHUR and JACKSON (1), indicating beyond doubt that a number of the known short-cycle forms are related to the corresponding long-cycle heteroecious species. As DIETEL points out, if the long-cycle forms were derived from the short-cycle ones, it would be expected that the latter would have a wider distribution than the former, because of their host relations. It seems hardly possible that a parasite dependent upon two unrelated hosts should have a wider distribution than one which can maintain its existence upon one host. Yet such is the rule, and leads us to the conclusion that the original form was the long-cycle heteroecious one, and that the reduced form has appeared only in certain restricted localities less favorable for the continued development of several spore stages.

4. CLIMATIC RELATIONSHIPS BETWEEN LONG AND SHORT-CYCLE RUSTS.—FISCHER (17) has presented some interesting data on the relative distribution of long-cycle and reduced species in Switzerland. Of the micro-forms, 53.7 per cent were found above the tree limit; of the lepto-forms, 27.3 per cent occurred above this limit; whereas of the eu-autoecious forms only 14.5 per cent occurred above the tree line, and of the eu-heteroecious forms only 17.9 per cent. MAGNUS (30) also pointed out the evident fact that the proportion of rusts with short cycles increases with the altitude. No comparable data are available in North America, but table I compares some short and long-cycle rusts belonging to *Puccinia*,

and indicates that there is a greater proportion of the long than of the short-cycle species represented in the tropics, and that a larger percentage of short-cycle species is represented in arctic regions. This indicates that the short-cycle species are more northern in distribution than the long-cycle ones, and possibly aligns the data with those of FISCHER in Switzerland on altitude.

TABLE I

DISTRIBUTION OF DICAEOMA AND MICROPUCCINIA

	TOTAL SPECIES	PERCENTAGE TROPICAL	PERCENTAGE TEMPERATE	PERCENTAGE ARCTIC
Long-cycle O, I, II, III...........	269	52.8*	60.0	2.9
Short-cycle III, or O, III........	149	34.8	71.8	10.7

* The fact that the total percentage in the three groups amounts to over 100 is occasioned by the overlapping of numerous species; for instance, a considerable number of species occur both in temperate and tropical America, and such were counted in both columns.

Another point that should be borne in mind is that the possession by long-cycle rusts of urediniospores as well as aeciospores, both of which are rather resistant to desiccation, and in addition are windborne for long distances, presumably enables them to be disseminated over wider areas. This feature, however, is limited by the extent of specific host specialization.

Morphological basis

The researches of KURSANOV (27) show that the structure of the aecidium, uredinium, and telium are essentially similar, in fact he terms them homologous; and that of these three sori, the first comes nearest to the fundamental type. The hypothesis is thus presented that because of the morphological similarity of these three structures, it is probable that the latter two must have been evolved from the most primitive, and have carried with them the morphological continuity of the aecial primordium.

The important point to be considered here, and one previously almost wholly overlooked or avoided, is whether this evolution took place before the time when the rusts became obligative parasites or after. No convincing arguments have been presented up to this

time showing that any short-cycle rust has evolved into a long-cycle form. OLIVE, CHRISTMAN, LINDFORS, GROVE, and others, who have taken the position that evolution from the short-cycle to the long-cycle types has thus arisen, have failed to take into account two important facts: (1) that progressive evolution is extremely rare among parasitic forms, and (2) that most of the types represented in their studies may be shown to have correlated long-cycle forms, which throws much skepticism on the value of their arguments. KURSANOV faces the situation squarely and comes to the conclusion (without entering into the parasitic argument) that the trend has been from the long to the short cycle by retrogressive processes. LINDFORS (28), while admitting this for certain species (*Uromyces acetosae*, etc.), concludes that the primitive type must have been short-cycle, and cites *Chrysomyxa abietis* as best representing the ancestral rust.

From the facts at hand there does not appear to be any evidence that progressive evolution has taken place in the ontogeny of any species of rust subsequent to our knowledge of this group. We are forced to agree with KURSANOV, therefore, that in the phylogeny of the rusts as such, all the convincing evidence points toward retrogressive changes, either by the omission of intermediate stages (aecia, uredinia, or both), or by nuclear fusion in the aeciospores, thus cutting off the vegetative sporophyte with its accompanying sori, to form the short-cycle types. The exact position of the first binucleate cells in these short-cycle forms is unimportant. The important feature here is that the telia hold the relative position in the cycle which was held by the aecia in long-cycle forms.

The types represented by *Endophyllum* and *Kunkelia*, most of which have known long-cycle correlatives, should not be disconcerting, since it is apparent that two sorts of abbreviation have taken place: (1) the types in which the telial stage has been drawn back to the position of the uredinial or aecial stage by the omission of either one or both of the intermediate structures; and (2) the types represented by *Endophyllum* and others, in which the soric cycle has been cut off at the end of the aecial stage and nuclear fusion takes place in the aeciospores, resulting in the formation of a basidium at the time of their germination. The fact that in certain forms

of *Endophyllum*, in *Kunkelia*, and in *Peridermium*, some of these spores do not produce basidia, but on the contrary produce germ tubes which may even reinfect the alternate host or the same host, as the case may be, does not furnish any evidence that the short-cycle forms are primitive and are thereby becoming long-cycle, but indicates that they are still more or less unstable and have not completely adopted the shortened cycle.

To return to the hypothesis, we find no evidence that short-cycle rusts are becoming long-cycle, but that some other explanation of the continuity of the aecial primordium must be substituted. We know that the rusts, like all other parasites, must have evolved from independent or saprophytic forms; and it seems most logical, therefore, to suppose that such morphological structures and homologies as exist between the various soral structures are merely reflections of their pre-parasitic condition, and more or less modified, of course, by their later environment.

Homologies with independent plants

Since it appears most logical to assume that the rusts are derived from an independent group of algal ancestors, we must study the comparative morphology of the rusts and the orders of algae. From the standpoint of the evident homologies existing between the several reproductive structures in the rusts and the red algae, coupled with dimorphism in the latter, we conclude that we should look to the red algae as the most logical group from which to derive the rusts.

The relationship which one group of plants holds with another group is based upon the homologues existing in the two groups. When both of such groups are independent plants the problem is difficult enough, but when any attempt is made to homologize the structural features of a dependent with a separate independent group, the problem becomes much more complex. The factors which parasitism injects into the problem cannot be stated in any accurate terms, and can only be approximated by a comparative study of the ontogeny of many individuals within both groups.

Without going further into the details of the evidence as regards the evolutionary tendencies among the rusts, I wish to point out that

all the evidence available at the present time appears to point to the conclusion that within the Uredinales the trend is toward increased physiological specialization, accompanied by morphological reduction through degeneration from the complex to the simple types; that is, from long cycle to short cycle by a shortening of the sporophyte. While this may seem anomalous to students of independent plants, I can come to no other logical explanation of the origin of our common short-cycle rusts. A similar reduction from complex to more simple morphological types is progressing in certain Ascomycetes, as indicated by the studies of FRASER (20) and others, pointing to the loss of oogonia and the suggestion of apogamous development. While there may be other reasons for this trend toward simplicity, the effect of parasitism on the parasite through physiological degeneration is apparently the chief contributing factor, and one which has been disregarded largely by those who have sought to build up a theory of phylogeny in this group of fungi.

Assuming that the primitive rusts were long-cycle, we are forced to search for homologies among those independent plant groups where an alternation of generations exists, comparable to that in rusts, and where pleomorphic spore forms are produced. Several writers, notably BLACKMAN (7) and YAMANOUCHI (42), have already pointed out the similarities between the rusts and the red algae, but none have analyzed this relationship as clearly as DODGE (15), who conceives the fusing cells of the aecidial primordia as merely intercalary. He states:

> The fusions are of exactly the same nature as those occurring between the accessory sterile and auxiliary cells in the red algae. . . . . There is no structure in the rusts which takes the place of or is the homologue of an egg apparatus. . . . . It is the lack of the element of femaleness represented by the egg which distinguishes a rust most fundamentally from its ancestral alga.

Furthermore, insufficient attention has been given to the significance of the teliospore together with its germination to produce the four basidiospores. Inasmuch as the teliospore with its basidium appears to be one of the most conservative (fixed) organs of the rusts, its importance in any scheme of phylogeny should be given adequate consideration. The outstanding features of the teliospore are that it terminates the sporophytic generation; it produces the basidium within which reduction divisions take place, with the for-

mation of four cells which generally produce four basidiospores initiating the gametophyte; and it is typically a resting spore with thickened walls. How do these features compare with the condition found among the red algae? In the Florideae the termination of the sporophyte takes place with the formation of the tetraspore mother cell. Within this cell reduction takes place, followed by the formation of four tetraspores which initiate the gametophyte. The tetraspore mother cell is usually thick walled, and frequently functions as a resting sporangium. It appears that the following homologies are indicated: the 1-celled teliospore is homologous with the tetraspore mother cell; the 4-celled basidium is homologous with the four tetraspores.

Whether there is much significance to be attached to the various types of teliospores among the rusts, in so far as they may indicate ancestral traits, is not clear. Certainly if we would search for a teliospore in the rusts which has the most striking likeness to tetrasporangia, we should cite the teliospores of *Uredinopsis*, *Hyalopsora*, *Milesia*, and other fern rusts. Among these may be found frequent examples of a tetrad arrangement of the teliospore cells, recognizing, of course, that the teliospores here are homologous with an aggregate of tetrasporangia. Perhaps of further significance is the lingering tendency observed among certain fern rusts to form their teliospores singly on the mycelium rather than in aggregates to produce sori.

The phenomenon of basidial formation within the teliospore so characteristic of the Coleosporiaceae should not be overlooked. The analogy here indicated with the tetrasporangium is most striking, and may indicate the most primitive conditions existing among the rust fungi. We should not overlook, of course, the possibility that the Uredinales are polyphyetic, and that the three families generally recognized are of separate origin. In the face of the argument of DIETEL (14) and others, that the Coleosporiaceae is a primitive group, it seems possible that this family may have been derived from a different line of ancestors, and is of more recent origin than the Uredinaceae. This might account also for the wider separation of the alternate hosts of the Coleosporiaceae, as well as the retaining of the characteristic formation of internal basidia.

As regards possible homologies with other spore structures of the

red algae, we must, of course, note the apparent relationship of the aecidium with the cystocarp. Without entering into the controversy over the morphological relationship between the red algae and the Ascomycetes, it appears pertinent to indicate, from KURSANOV'S (27) studies, that the aecidium is to be considered the primary fructification of the rusts, and that striking similarities occur between this organ and the cystocarp. The fact that in both of these organs the sporophyte is initiated from the gametophyte is fundamental. While it is true that in most rusts nuclear fusion is delayed, it appears probable that the present hyphal conjugation is to be regarded as a process which has arisen through degeneration as a substitute for some more elaborate process, such as exists in the red algae. The reasons for such degeneration may be attributed to the acquisition of the parasitic habit without overworking the idea of degeneration through parasitism.

While we cannot with certainty homologize the fertilized carpogone with the fusion cells of the aecidium, it is apparent that the carpospores are homologous with the aeciospores. Other relationships are suggested, but must await more detailed studies.

The question also arises regarding the origin of the uredinia, with their very characteristic echinulate urediniospores. One might be inclined to assume a relationship between urediniospores and the monospores of the red algae, but so little is known regarding the latter that it is hardly possible at this time to make further suggestion.

Of special significance is the discovery of HOWE (22, 23) that dimorphism exists among the red algae, and that the carpospores of *Galaxaura* undoubtedly produce on germination a structurally distinct sporophytic plant, which in turn bears tetraspores which eventually complete the dimorphism by producing the gametophytic plant. This condition is not unique for the red algae, since SAUVAGEAU (40) has established a similar condition in the Laminariaceae of the brown algae, and it seems probable that eventually we shall find this condition rather common, at least among the red algae. It is not improbable, as has been suggested by others, that a number of the red algae known only in the carposporic or tetrasporic stages represent one stage only of dimorphic species.

It would appear that HOWE's discovery very probably bears directly on the relationships which are apparent between the red algae and the rusts. If we consider the ancestors of the rusts to have been independent algal plants, we could not hope to point out a closer relationship than is now apparent between the dimorphic Florideae and the heteroecious rusts. The life cycles are as nearly identical as can be expected to exist between two groups, one independent and the other dependent.

The view of C. E. BESSEY (5), E. A. BESSEY (6), and others, that the rusts originated from the Ascomycetes, seems highly improbable in the face of all the evidence presented. There would seem to be no convincing analogy between the two most fixed organs of these groups, that is, the teliospore and the ascus; neither is there any very close relationship between the aecium and the ascus. If the rusts had been derived from the Ascomycetes, we should certainly expect to find more striking structural similarities between the two groups, and especially between the parasitic representatives of the Ascomycetes and the rusts. The absence of any real significant structures common to Ascomycetes and the rusts leads to the conclusion that the former had their origin from a different order of plants, or at least from a different group of the red algae than that giving rise to the rusts.

FITZPATRICK (18, 19), in his studies on *Eocronartium muscicola*, brought forward the hypothesis, earlier suggested by ATKINSON (3), that the Uredinales may have arisen from forms similar to those now included in the Auriculariaceae, on the basis of the apparent parasitism of certain members of this group, but more especially on the evident homology between the cell of the teliospores in the rusts and the cell giving rise to the basidium in the Auriculariaceae, and the general similarity between the cross walled basidia of the two groups. While these similarities must be conceded, there appears to be little else in common between the rusts and the Auriculariaceae. In so far as the investigations on this last group have progressed, there is no evidence of a fixed alternation of generations, and particularly of any such definite and outstanding feature as the haploid mycelium in the rusts. It would appear more logical to grant a similar origin to the two groups, as indeed practically all mycologists

have done for the Basidiomycetes as a whole, but without acknowledging for the present the evolution of the one from the other.

Significance of host relationship of heteroecious rusts

Since what appear to be the most primitive rusts inhabit ferns and a number of the Pinaceae (*Abies*), it seems probable that these plants represent the original hosts of the Uredinales, and that heteroecism was established on them. The phenomenon of heteroecism is found throughout the Uredinales and nowhere else among the fungi, unless we admit certain species of Ascomycetes (*Sclerotinia heteroica*). That this remarkable condition should be so strictly limited to a single order of fungous parasites indicates that it is of significance as regards the origin of the group. If this phenomenon was correlated with any definite host relationship, its origin might be explained on other grounds, but all students of the rusts have commented on the lack of any apparent relationship between the alternate hosts of the heteroecious rusts. If heteroecism arose by the sudden jumping of one stage of a rust to some other unrelated host, as some workers believe, or if it arose through the gradual restriction of hosts from a plurivorous condition, as others are inclined to think, we are led at once to question why the rusts have so exclusively developed this habit.

On the grounds of pleomorphy, parasitism, and purivory, there is no apparent reason why heteroecism fails to appear more generally in the Ascomycetes, and also in the Oomycetes and the higher Basidiomycetes. Its failure to appear in these other groups may indicate that they had a different origin from that of the rusts. Surely if the Ascomycetes and the rusts both originated from the Florideae, we should expect to find in the former some indication of this phenomenon of heteroecism so outstanding in the latter. Even though the impossibility of separating the ascocarp from the gametophyte precludes the establishment of heteroecism in the Ascomycetes on the basis of the alternation of generations, still heteroecism is not necessarily dependent upon such a separation. It would appear, therefore, that some fundamental difference existed between the ancestors of the rusts and the Ascomycetes which has been the chief factor in determining heteroecism in the former. Such a differ-

ence may possibly have been dimorphism of the sort previously discussed.

While we admit that little, if any, relationship exists between the alternate hosts of heteroecious rusts, we find in certain genera, especially of the Uredinaceae, a closer relationship between such hosts than is shown in the Coleosporiaceae and Aecidiaceae. It will be worth while to point out here that among those genera of rusts which are parasitic on ferns (*Uredinopsis*, *Hyalopsora*, and *Milesia*), the alternate host in each case is a species of *Abies* belonging to the Pinaceae. Nowhere else do we find so close a relationship between the alternate hosts of rusts. As already pointed out, there are excellent indications that these particular rusts are primitive, as indicated by the structure of the teliospore and in numerous instances its poorly defined sorus. If such a supposition should prove to be the case, we might hypothecate that the rusts originated as such in a period typical of ferns and conifers, and that these two groups represent the primitive hosts of the Uredinaceae. ARTHUR (2) goes further and suggests that the fern rusts represented by *Desmella* are primitive forms of Pucciniaceae. This particular genus inhabits ferns belonging to the Polypodiaceae and Schizacaceae, which are presumably of more recent origin than the Osmundaceae, which harbors *Uredinopsis* and related rusts. It is not improbable, therefore, that both heteroecism and autoecism developed in such a period, and that the segregation of the rusts took place during such an early time.

Correlations between various species of rust

Correlated species of rusts are most prevalent in *Puccinia* and *Uromyces*, which have been considered the more recently evolved types. Since many of these correlations involve short-cycle forms, additional evidence is adduced that primitive rusts are long-cycle.

No attempt will be made here to present a list of correlated species, but rather, by the following grouping, to show what sort of correlations exist, and point out a few significant points which seem to bear on the origin of certain species. All of the following examples are taken from the two largest genera of rusts, *Uromyces* and *Puccinia*. Parallel correlations are to be found throughout the

order, but not so frequently as between species belonging in these two genera.

From a study of the following cases it is evident that at least three different types of correlation are present, as follows: (1) Correlations between autoecious and heteroecious species of eu-*Puccinia* and eu-*Uromyces* in which the only difference lies in the number of cells in the teliospore. From the fact that in general the 1-celled form is less widely distributed, and as the writer has remarked (36), appears less vigorous than its correlated 2-celled form, it appears most logical to suppose that the 1-celled race was derived from its corresponding *Puccinia*. If this is true, here again we have evidence of degeneration taking the form of dropping one cell from the teliospore. The presence of mesospores in the telia of many species of *Puccinia* further indicates this trend. (2) Correlations between heteroecious eu-*Puccinia* and micro or lepto-*Puccinia*, in which the latter develops telia on the gametophytic host of the former. These correlations are very common, and have been known since TRANZSCHEL noted the classical case of *Tranzschelia punctata* and *Polythetic fusca*, and DIETEL (9) mentioned the case of *Puccinia mesneriana* and *P. coronata*. (3) Triple correlations between autoecious species of eu, opsis, and lepto or micro-*Puccinia* which have been discussed briefly by the writer (37), who believes the opsis-forms to be intermediates between short and long-cycle autoecious rusts. This is indicated by the rather frequent presence of urediniospores in the telia of the opsis forms, and aeciospores in the telia of the micro and lepto-forms, indicating that uredinia disappear first and aecia later.

All of these correlations seem to be explained most clearly on the basis of the simpler forms having been derived from the more complex, by reduction or the dropping out of some structure or stage. That this reduction is not confined to the sporophyte is evidenced by the fact that a large proportion of the reduced forms have also dropped the pycnial stage and are now represented by telia only.

Effects of evolutionary progress of host upon host relations

It seems quite possible that the phylogeny of the plants which now serve as hosts of heteroecious rusts had an important bearing upon their present wide separation in the system of classification.

It seems unlikely, as DIETEL (11) points out, that a group of obligate parasites, like the rusts, could have passed through the many evident changes which have taken place and are still continuing, without being affected by the evolutionary processes of their host. While the antiquity of the rusts (relative to the higher plants which serve as hosts) is unknown, it seems probable that they date back at least to a period where phylogenetic lines in the higher plants were being evolved.

If we should ever be so fortunate as to establish the origin of parasitism in the rusts before or at the time of the supposed origin of the phylogenetic lines derived from the Ranunculaceae, we should have to postulate an association which has since that time passed through many vicissitudes, during which some less fortunate groups have been left behind, and the more fortunate ones evolved into our higher types of vegetation. Is it not probable that during this progress in the evolution of the higher plants the segregation of the rusts has gone on in a similar manner? For instance, why do we find a unique genus like *Uromycladium* confined solely to one genus of host plants as *Acacia?* In the same way, why is *Ravenelia* confined to Mimosaceae and close relatives? While the writer does not propose to make a thesis of this point, it certainly seems probable that we have had a wide segregation of the rusts coincident with their parasitic development. May it not be possible that the wide separation of the gametophytic and sporophytic hosts, as in the case of many heteroecious rusts, has come about through the more rapid evolution of one host than the other?

Significance of antithesis between gametophyte and sporophyte in light of physiological responses produced by each on their host plants

The antithetic responses produced by the alternate phases of rusts indicate their origin from ancestors in which the gametophyte and sporophyte were vegetatively independent of each other.

In studying the pathological responses produced by rusts an interesting fact has appeared, that is, that with very few exceptions the most outstanding deformities are produced in the host by the gametophytic mycelium. This is evident in both heteroecious and

autoecious species. Two or three typical examples will serve to illustrate the point. *Uromyces proeminens*, an autoecious long-cycle rust, produces in its gametophytic stage a conspicuous change upon its host *Euphorbia*. In this stage the rust is systemic and causes etiolation and an upright habit of growth of its host, accompanied by leaf dwarfing. In the sporophytic stage the rust is not systemic and does not cause any pronounced symptoms. A very similar effect is produced by a group of heteroecious rusts well represented by *Uromyces pisi*, having their gametophytic mycelium in *Euphorbia* and the sporophytic in Fabaceae. Marked changes in form of host occur in *Euphorbia*, but nothing noteworthy in symptomatic effects is produced by the sporophyte. A considerable number of short-cycle rusts also show this particular type of symptom. A typical example is *Puccinia holboellii*, a short-cycle rust on *Arabis*, which is systemic, producing dwarfing and sometimes a witches' broom effect. Its long-cycle correlated species *Puccinia monoica* bears its gametophytic stage on *Arabis* and its sporophytic on two grasses, *Koeleria* and *Trisetum*. In the former phase the same symptoms are produced on *Arabis* as are produced by *Puccinia holboellii*. The species of *Cronartium* present an analogous condition. The gametophytic stage on *Pinus* produces galls and swellings of various sorts, while the sporophyte produces no such effects. These phenomena indicate that the effects produced by the gametophyte of a short-cycle rust are comparable with the effects produced by the same mycelial generation of a long-cycle rust, irrespective of the associate spore stages. There is, then, not only a fundamental morphological distinction between the gametophyte and the sporophyte, but the distinction is linked with fundamental physiological differences in effects produced upon their hosts.

These significant antithetic effects produced independently by the two stages of rusts can hardly be due to host relations, or to the types of tissue invaded by the respective phases. It must be more fundamental, and it would appear logical to trace it back to a separate origin for each stage, such as would be expected if these rusts originated from dimorphic ancestors similar to the condition now known to occur in the red algae.

Summary

1. Little attention has been given to the significance of the haploid and diploid generations in the rusts, in the light of their similarity to the alternate generations in the algae on the one hand, and their correspondence with the heteroecious habit of the rusts on the other hand. BLACKMAN calls particular attention to the close analogy with the red algae in discussing the relationships of the Uredineae, and YAMANOUCHI draws a similar analogy in his discussion of the relationship of one of the red algae, *Polysiphonia*, with the rusts. Other writers also have mentioned this analogy, but have not made a point of the separation of the generations corresponding with the heteroecious habit. It is certainly significant that the antithetic generations of mycelium are invariably separated on unrelated hosts in all heteroecious Uredinales.

2. While the structural similarities existing between the rusts and the red algae are striking, it appears that they are supported by certain homologies between the two groups. The teliospore is homologous with the tetrasporangium, the basidium with the four tetraspores, and therefore the gametophytic thalli of the two groups are homologous. Since the pycnia of rusts and the male conceptacles of red algae are borne on the gametophyte and show striking similarities in structure, it seems possible that they are homologous, in which case the pycniospores are homologues of the spermatids. So far as the evidence has been presented, it appears that the aeciospores of rusts may be homologous with the carpospores, and since both produce the vegetative sporophytes, the latter are homologues. Of further significance is the close analogy between the functions of the corresponding homologous organs. It would be difficult to find a closer apparent relationship between two groups of plants, one independent and the other dependent.

3. Our knowledge of fossil rusts is very meager, but of the few species described, only aecial and telial stages are known. This certainly indicates that these are the most conservative organs of the rusts, and this is borne out by our knowledge of present day rusts. We may conclude, therefore, that primitive rusts possessed both the aecial and telial fructifications.

4. The trend of phylogeny in the rusts is indicated by a comparative study of their life cycles. The relative numerical importance of long and short-cycle rusts is greatly in favor of considering the short-cycle forms as arising by reduction from the long-cycle species. The short-cycle rusts constitute less than 20 per cent of the total species; they have a much more restricted geographical distribution as well as climatic adaptation; and a large number of them are already known to be definitely correlated with long-cycle species, which certainly appear to possess the more primitive characters. In addition to these points, the proportion of long and short-cycle species of *Puccinia* inhabiting the Campanulales is about one to two, indicating, as KERN (24) has pointed out, the probability that the short-cycle forms are the more recent. The fact that reduction has taken place is clearly shown in the case of *Kunkelia nitens* being derived from *Gymnoconia interstitialis.*

5. The acquisition of the parasitic habit invariably results in degeneration, as indicated by the loss of independence through the disappearance of chlorophyll and chromatophores, a reduction in the size of the thallus, and probably a reduction in its structural differentiation. The evidence is all in favor of reduction in morphological structures through the loss of uredinia, aecia, and pycnia, as well as reduction in the number of cells in the teliospore. An increasing host specialization indicates still greater dependence in food relations which can only be interpreted as tending toward further degeneration and reduction to the simplest types which produce telia only.

6. The importance of the host relations of heteroecious rusts is of special significance, because this phenomenon is characteristic only of this order of parasitic fungi. If heteroecism arose by a sudden jump from one host to another, it is curious that it has not appeared in such groups as the Oomycetes, Ascomycetes, and the smuts; and further, we should expect the jump to have been made in some cases to closely related hosts. Since the evidence is all negative from these standpoints, we must conclude that the phenomenon of heteroecism has a different origin. If the heteroecious habit was derived from the autoecious by a gradual restriction of the two generations to separate hosts, we should again expect to find heteroecious rusts passing

their alternate stages on closely related plants, at least in some cases. The evidence is also negative regarding this point, and we are forced to conclude that heteroecism is an ancient feature of the group.

7. The existing correlations between heteroecious and autoecious species, coupled with correlations between long and short-cycle species, all point to the heteroecious and long-cycle autoecious species as being the most primitive. We can agree with FISCHER that the primitive rusts were probably plurivorous, but we cannot subscribe to his ideas as to the manner in which heteroecism arose. FISCHER'S ideas regarding the origin of the short-cycle forms seem sound in general, and DIETEL'S recent views regarding the origin of the short-cycle forms from the heteroecious species appear logical in the light of the evidence as he has presented it, and as we conceive the phenomenon. There would seem to be no reason, however, why he should retain the idea of host changing in special cases by sudden jumps, as, for instance, in the case of the fern rusts, which according to him appear to have transferred their gametophytic generation to *Abies*, or why he should believe that a similar sort of change took place in *Melampsora*. In the same way we must reject the proposals of KLEBAHN, OLIVE, and others who have postulated the jumping of the sporophytic stages to a separate set of hosts.

The theories of BARCLAY and GROVE regarding *Endophyllum* are more logically explained on the ground that this genus represents a reduced type similar to *Kunkelia nitens*, which has been derived simply by the fusion of the nuclei in the aeciospore of a former eu-heteroecious *Puccinia*.

8. The confinement of certain unique genera like *Uromycopsis*, *Uromycladium*, *Ravenelia*, etc., to certain host families or genera indicates the probability of the early establishment and segregation of these rusts on particular branches of the phanerogamic tree. I wish to raise the question whether the wide gaps between the alternate hosts of certain rusts may not have been accentuated through the processes of evolution of the particular phanerogams which they inhabit?

9. The antithetic effects produced upon their respective hosts by the gametophyte of the rusts, which is evident in both autoecious and heteroecious species, indicate a primitive character as well as a

dimorphic condition of their ancestors, similar to that which HOWE has described for *Galaxaura*.

10. I conceive the rusts as originating from dimorphic red algae after the following manner. An endophytic tendency resulted in the gametophyte and sporophyte independently adapting themselves to whatever hosts were present. Postulating *Uredinopsis* as an ancient type, we may conceive of the independent sporophyte gaining a foothold in the ferns and the gametophyte establishing itself in the Abietineae. As parasitism became more fixed, the specialization to certain species of ferns and Abietineae became more complete, and heteroecism was established. I am thus in agreement with BLACKMAN regarding heteroecism as being a primitive character of the Uredinales. There appears to be no objection to supposing that in numerous cases both the gametophyte and sporophyte selected the same host, resulting in autoecism. In the light of such a hypothesis I can see no objection to deriving the short-cycle forms from long-cycle heteroecious and autoecious ancestors. The presence of correlated short-cycle forms of both heteroecious and autoecious species is our best evidence that such has taken place. The opsis and brachy types appear generally to represent intermediate forms between eu-autoecious species and their short-cycle micro and lepto-forms, since a number of such correlations are known. We should, therefore, search among the dimorphic red algae, and especially those forms which are parasitic, for evidences of the development of rust-like features, such as the basidium, the loss of sex organs, and a pleophagic condition of the alternate generations verging toward heteroecism.

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A SECOND CASE OF MATERNAL INHERITANCE OF CHLOROPHYLL IN MAIZE[1]

M. DEMEREC

(WITH ONE FIGURE)

A character inherited only through the female gametes in maize has already been described by ANDERSON (1). He showed that the striped plants gave green, striped, and pale green progenies, whether pollinated with the pollen from the green or the striped plants. The green plants obtained from the striped ones gave only green progeny, the striped plants repeated the performance of the parent, and the pale green plants died. When seeds from striped plants were planted in the same order as found on the ear, the distribution of different types was not at random, but similar ones were grouped in patches. A test of pollen from striped plants showed that the character was not transmitted through the pollen. The case which will be described in this paper duplicates in all respects the inheritance of the character described by ANDERSON. It differs from it, however, by having an independent origin and a dissimilar phenotype.

Material

The origin of the character dealt with in this paper was from a single variegated plant found by Professor C. B. HUTCHINSON in a field of Lucis Favorite maize grown for commercial purposes on Long Island. Seeds from this plant, which was open pollinated, were harvested when ripe, and given to the writer for genetical studies. A planting of these seeds made in the greenhouse gave 51 green, 26 pale green, and 48 variegated seedlings, among which last 3 were very light variegated (almost pale green), 26 light, 13 medium, and 6 very dark variegated. These results indicated that the newly found character might not be Mendelian in inheritance, so that the experiments were planned from the beginning in such a way as to test this hypothesis.

[1] Experiments reported in this paper were completed in the Department of Plant Breeding of Cornell University, Ithaca, New York.

Description

Among the progeny of a variegated plant three kinds of seedlings may be found, green, variegated, and pale green. The pale green seedlings are typically expallescent, that is, in spite of having a large amount of chlorophyll they die in the early seedling stage (DEMEREC 15). An analysis of the chlorophyll content was made according to the method described in this earlier paper, and it was found that pale green seedlings in three analyses made with different material had 46, 50, and 55 per cent of the amount of chlorophyll found in green plants of the same family. Pale green seedlings invariably died in approximately the same stage of development in which white seedlings die.

In the case of the variegated seedlings, if the green portions are small they die; but if the green portions are large enough for adequate production of food they develop into variegated plants. The amount of chlorophyll found in the pale green parts of the leaves decreases with the age of the leaf. Examination of a half-grown variegated plant shows that the upper leaves are pale green and green variegated, and that on the lower ones the pale green portions have become lighter and lighter, until they reach a yellowish white appearance, with little if any green color. A fully developed variegated plant looks like yellow and green, and not pale green and green variegated. A color determination was made on a variegated plant at the tasselling stage, using RIDGWAY'S (27) color standards, and it was found that green parts were elm green 27-m; and the pale green parts, from the upper leaves toward the lower, were as follows: light cress green 29″-i, lime green 25″, reed yellow 23″-b, and straw yellow 21′-d. A chlorophyll analysis of the leaves, taken from a variegated plant of the same stage, showed that the pale green portion of the upper leaf had 66.6 per cent and the pale green portion of the lower leaf 7.3 per cent as much chlorophyll as the green portions taken from the same leaves. So far as observed, the green seedlings always developed into green plants.

Independence of maternal types

The surest and simplest method to establish a genetic difference for two Mendelian characters, which are phenotypically similar, is

to intercross them. In the case of characters which do not behave in the regular Mendelian way, however, this method fails. When deal-

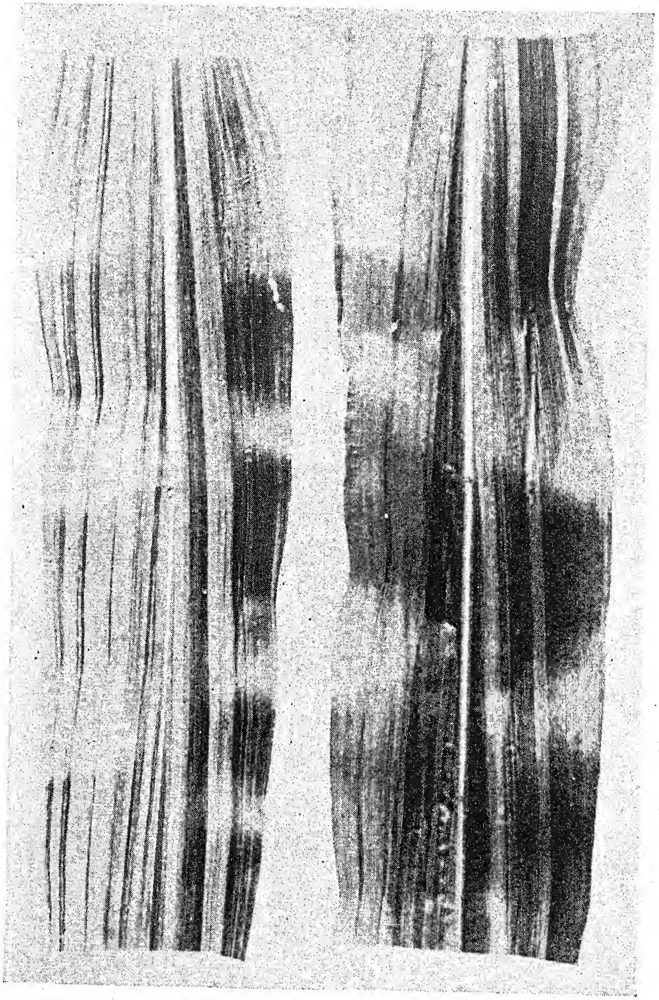

FIG. 1.—Leaves from variegated plant

ing with such characters, similar in effect, there is no way of determining with certainty whether they are genetically different or not.

In the present case it is possible to show that the maternal type described in this paper originated independently from the type de-

scribed by ANDERSON. This latter arose as a single plant, at Cornell University, in ANDERSON's cultures for genetic studies. On the other hand, the type dealt with in this paper was found as a single plant in a field of maize on Long Island, about 300 miles from Cornell. So far as known there is no evidence to indicate that the lines where these two types were found were ever intercrossed, or that the seeds have been mixed. This second maternal type was found in Lucis Favorite variety, which differs morphologically and in several known genetic characters from the variety in which the first maternal type was found. Subsequent breeding tests, carried on for several generations, showed that all progeny from the original ear taken from the first variegated plant were typically Lucis Favorite. This eliminated any possibility of intercrosses or mixture of seeds, and made it very probable that the two maternal types were independent in origin.

In addition to being independent in origin, the second maternal type also differs phenotypically from the first one. This difference is especially pronounced in the seedling state or in the young variegated plants. The pale green color of ANDERSON's maternal type is much lighter than that of mine. An analysis of the chlorophyll content made with the material, grown at the same time and under the same conditions, showed that the pale green seedlings of ANDERSON's maternal had 6 per cent of the normal chlorophyll, while the pale green seedlings of my maternal type had 46 per cent. A similar difference in the intensity of the pale green color can also be noticed on variegated plants when young. When variegated plants grow older that difference disappears, however, and both types look similarly yellow-white and green variegated. A photograph of the full grown variegated leaves of my maternal type is shown in fig. 1.

Inheritance

TESTS OF FEMALE GAMETES.—As already mentioned, variegated plants produce three kinds of progeny, green, variegated, and pale green. Since the pale green plants die in the early seedling stage, only green and variegated plants could be used in the experiments to test the inheritance of the character.

PROGENY OF GREEN PLANTS.—So far as tested, green plants gave nothing but green progeny. From five different lines twenty-nine

green sibs of variegated plants were self-pollinated, and 4840 seedlings, grown from the seed of these plants, were examined. All of them were green, showing that the green plants did not transmit the variegation.

PROGENY OF VARIEGATED PLANTS.—Variegated plants rarely gave only green progeny, but usually produced pale green, variegated, and green progeny in varying proportions, irrespective of the kind of pollen used in fertilization. To determine the effect of the foreign pollen, the fertilization was brought about with a mixture of pollen from the variegated plant which was to be pollinated, and from some unrelated green plant which carried the dominant endosperm character present in the variegated plant in a recessive condition. On an ear pollinated by such a mixture of pollen it was possible by xenia to distinguish the seeds which were the result of self-pollination from those which came from the cross. This made it possible to determine on the same ear the effect of different male gametes on the progeny of a variegated plant. The data on the progeny of nine variegated plants pollinated with a mixture of pollen are given in table I, from which it can be seen that the male gametes did not influence the type of the progeny obtained. On individual ears the relation between the number of pale green, variegated, and green seedlings was practically the same from selfed and from crossed seeds.

TABLE I

COMPARISON BETWEEN PROGENIES OF SELFED AND CROSSED SEEDS TAKEN FROM SAME EARS

PEDIGREE NO.	PROGENY FROM					
	SELFED SEEDS			CROSSED SEEDS		
	Pale green	Variegated	Green	Pale green	Variegated	Green
613–19	....	....	115	....	....	3
617–30	....	....	70	....	....	4
617–27	....	....	65	....	....	3
613–22	0	3	104	5	11	11
613–5	11	15	46	18	10	14
613–7	63	20	15	61	14	17
617–1	190	27	14	23	11	3
613–10	186	7	3	25	2	5
459–2	139	8	2	5	....	....

Table II gives the progeny of sixteen variegated plants, which are arranged in three groups according to the intensity of variegation. From this table it can be seen that plants with large pale green areas are likely to give more pale green and variegated, and less green progenies, than plants where the pale green areas are very small. Some of the plants with few pale green stripes, however, gave

TABLE II

PROGENY OF VARIEGATED PLANTS

Pedigree no.	Appearance of ♀ parent	Type			Percentage		
		Pale Green	Variegated	Green	Pale green	Variegated	Green
617–29....	Dark variegated (pale green parts take less than ¼ leaf area)	0	0	80	......	......	100.0
617–29....		0	0	68	......	......	100.0
613–5.....		29	25	90	20.2	17.4	62.4
617–10....		28	30	50	25.9	27.8	46.3
459–7.....		55	79	76	26.2	37.6	36.2
	Total..............	112	134	364	18.4	22.0	59.6
617–30....	Medium variegated (pale green parts take ¼–½ leaf area)	0	0	74	......	......	100.0
613–19....		0	0	118	......	......	100.0
617–30....		0	0	74	......	......	100.0
613–7.....		124	34	32	65.2	17.9	16.9
617–1.....		213	38	17	79.4	14.2	6.4
613–10....		211	9	8	92.5	4.0	3.5
	Total..............	548	81	323	57.5	8.5	34.0
613–22....	Light variegated (pale green parts take more than ½ leaf area)	5	14	115	3.7	10.4	85.9
613–23....		1	7	5	7.7	53.8	38.5
617–17....		29	43	21	31.2	46.2	22.6
459–2.....		144	8	2	93.5	5.2	1.3
613–2.....		25	0	0	100.0	......	......
	Total..............	204	72	143	48.7	34.1	17.2

relatively more pale green and variegated seedlings than those which had large pale green stripes, showing that the type of progeny does not always depend on the type of variegation of the parent plant. It is probable that the type of the progeny was determined by the type of tissue in the region where the ear was located, but no observations were made to prove this.

As in the case described by ANDERSON, it was found here also that the distribution of different types of seedlings with respect to their

position on the ear is not random, but that there was a tendency of like types to occur in patches. This can easily be observed by planting the seeds in approximately the same order as their location on the ear. Table III gives the results of one of the several plantings made. In this case the plant was pollinated with a mixture, and all seedlings resulting from cross-pollination are indicated in italics.

TABLE III

DIAGRAM OF EAR FROM VARIEGATED PLANT POLLINATED BY MIXTURE, GIVING APPROXIMATE POSITIONS OF SEEDS PRODUCING GREEN (*g*) VARIEGATED (*v*), AND PALE GREEN (*p*) SEEDLINGS; ITALIC TYPE INDICATES CROSS-POLLINATED SEEDS AND ROMAN TYPE INDICATES SELFED SEEDS

SEED NO.	Row					
	First	Second	Third	Fourth	Fifth	Sixth
1	*p*	v	*p*	*p*	*p*	p
2	*p*	*v*	p	*p*	p	p
3	*v*	v	p	*p*	p	*p*
4	*v*	p	*p*	p	*p*	p
5	p	p	p	*p*	*p*	p
6	p	*p*	p	p	*p*	*p*
7	*v*	v	p	p	*p*	p
8	p	v	*v*	*p*	*p*	p
9	*p*	p	p	g	*g*	p
10	p	p	*p*	g	v	p
11	v	*v*	*p*	*v*	v	*p*
12	*v*	p	p	*g*	v	p
13	v	*p*	p	g	*v*	*p*
14	*v*	p	*v*	*g*	v	p
15	p	p	*p*	g	v	*p*
16	p	*g*	*p*	p	v	*p*
17	g	g	*p*	p	*p*	p
18	*g*	*g*	*g*	*p*	p	*p*
19	g	g	g	*p*	v	p
20	....	g	*g*	*g*	*p*	*p*

TESTS OF MALE GAMETES.—All experiments made with pollen from the variegated plants showed that the male gametes do not transmit the variegation to the progeny. With the pollen taken from nine variegated plants of different intensity, ten green plants were pollinated. The 651 F_1 plants were examined in the seedling stage, and 75 of them were grown to maturity, all being normally green. Fifty-six F_1 plants were selfed and 16,675 F_2 seedlings examined, and none of them was either variegated or pale green. Several ears segre-

gated virescent seedlings in a 3:1 ratio, however, showing that one of the parents brought into the cross a gene for virescent seedlings.

Inoculation experiments

To test the possibility of the variegation being caused by a virus disease, inoculation experiments were made with young seedlings and with older plants. The method of inoculation was the same in both cases, namely, pale green leaves taken from the seedlings of the maternal line were rubbed thoroughly with the green leaves of the plant being inoculated. The injury done to the inoculated leaves differed on different plants, from a very slight to an extensive one. Inoculation by grafting was not tried, since grafting is rarely if at all successful in maize.

EXPERIMENT WITH SEEDLINGS.—About 350 green seedlings were inoculated at the four-leaf stage, and were kept in the greenhouse until the plants began to tassel. They were examined several times during their growth without any effect of the inoculation being noticed. All the plants, in all the stages at which the examination was made, were uniformly green.

EXPERIMENTS WITH MATURE PLANTS.—Mature plants were inoculated at the tasseling stage, inoculation being made on three leaves on each plant, namely, on the youngest and two of the older ones. In the experiment forty-four plants were used, seven of them being green sibs of variegated plants, and the remaining thirty-seven from crosses in which the male parent was a variegated plant. Careful examination of the inoculated plants was made several times, but no indication was observed of variegation being transmitted. From twenty-nine inoculated plants which were self-pollinated, 8576 seedlings were grown, and all of them were green.

Conclusions

Experiments made to test the inheritance of this variegation showed that it is inherited only through the female gametes, and that the male gametes have no influence either on its transmission or its expression. All attempts made to transmit the variegation asexually were unsuccessful.

Discussion

Among the characters affecting the development of chlorophyll in plants, many have been described which do not follow regular Mendelian inheritance. In regard to their transmission they can be divided into two groups (SHARP 28), namely, characters having a maternal inheritance and those inherited biparentally.

Chlorophyll variegations inherited only through the female parent were described in *Antirrhinum* (BAUR 2), *Arabis* and *Aubretia* (CORRENS 11), *Glycine* (TERAO 33), *Humulus* (WINGE 34), *Hydrangea* (CHITTENDEN 6), maize (ANDERSON 1 and the writer in the present paper), *Melandrium* (SHULL 29), *Mesembryanthemum* (CORRENS 11), *Mimulus* (BROŽEK 5), *Mirabilis* (CORRENS 9, 10), *Primula* (GREGORY 18), and *Stellaria* (CORRENS 13). Not completely tested, but probably belonging to the same group, are variegations described by CORRENS (12, 13) in *Hieracium*, *Mercurialis*, *Senecio*, and *Taraxacum*, and by KAJANUS (21) in *Pisum*.

The typical genetic behavior of the maternal variegations is as follows. (1) Seeds from the green branches of a variegated plant give green progeny, those from the pale green or white branches give pale green or white progeny respectively, and the seeds from the variegated branches give variegated, green, and pale green (or white) progeny in different proportions. (2) When a variegated plant is pollinated with the pollen from a green plant, the F_1 offspring do not differ from the offspring resulting from a selfing. (3) Pollen from the variegated plants does not transmit the variegation on F_1, F_2, or F_3 progenies.

In explaining the unusual inheritance of maternal variegations, CORRENS (9, 10) assumed that the character is not transmitted through the nucleus, since the sperm nucleus from the variegated parent does not transmit variegation, nor does the sperm nucleus from the green parent produce any change in the type of the progeny of a variegated plant. He thinks that the inheritance of these variegations is carried through the cytoplasm. With slight modifications, CORREN'S hypothesis is almost universally accepted among geneticists. Some of them, however, are more specific than CORRENS in assuming that the bearers of inheritance in such cases are the plastids (GREGORY 18, WINGE 34, CHITTENDEN 6).

An attempt was made by STOMPS (31) to explain such maternal inheritance on a chromosomal basis. The exceptional results, obtained in the reciprocal crosses between a maternal character and the type, he thinks have a parallel in certain crosses of species of *Oenothera*. Since the characters so inherited in *Oenothera* are assumed to be carried in the chromosomes, he does not see the need for another mechanism in explaining the heredity of maternal types.

In the case of maternal variegations, the experimental evidence in support of any of the explanations of their inheritance is not sufficient to be considered conclusive. There are no adequate F_2 data available from the cross between variegated female and green male. Such data obtained from the seeds grown from the light colored regions of variegated F_1 plants would complete the tests as to the effect of the male nucleus on the inheritance of these variegations. As the evidence stands now, it is possible to explain all of the observed facts by the assumption that the variegation is determined by a dominant mutable gene which has a highly increased frequency of mutations (as compared with the gene for the type), when it comes in contact with other than parental cytoplasm. Such a gene would become more mutable in the crosses between the type and variegated when the pollen from a variegated plant was used. If the rate of mutation from a gene for white or pale green to a gene for the type is high enough, the character would disappear in the early stages of development of the F_1 plants, and the young seedlings as well as the mature F_1 plants would be green, both somatically and genetically. On the other hand, in the case of the opposite cross (variegated ♀ by green ♂), the rate of mutation in the gene determining the character would not be increased, because the gene would be in the parental cytoplasm. Such a cross would be expected to give results similar to those obtained by self-pollination.

This maternal inheritance could also be explained by balanced lethals, as suggested by STURTEVANT (32). There is also a case in *Oenothera* described by KLEBAHN (22)[2] which duplicates the behavior of these maternal characters, and which would indicate that the nu-

[2] The writer is indebted to Dr. STURTEVANT for calling his attention to KLEBAHN'S paper.

clear mechanism not only could, but does account for the unusual behavior of characters inherited through the female gametes only.

As already mentioned, the experimental evidence does not justify a final conclusion on the mechanism for the inheritance of maternal variegations. In addition to the cytoplasmic explanation, there are several possibilities of explaining the observed facts on a chromosomal basis. It might be well to defer judgment as to which of the explanations is most probable, until more experimental evidence has been accumulated. The fact, however, that all known maternal characters deal with the development of chlorophyll, favors the non-Mendelian explanation, since it was shown by RENNER (26) and DAHLGREN (14) that chloroplasts may act as bearers of the inheritance of chlorophyll characters.

The variegation in *Pelargonium zonale*, which was genetically studied by BAUR (2) and NOACK (24, 25), is the only case described in which inheritance is biparental.[3] The variegation in *Capsicum annuum*, as described by IKENO (19, 20), might belong to the same group. Recent investigations, however, the results of which the writer learned from a conversation with Professor IKENO, indicate that the variegation in *Capsicum* is the result of an infectious disease.[4]

BAUR'S data on the inheritance of *Pelargonium zonale* are given in table IV, from which it can be seen that the selfing of a *zonale* plant gave only white progeny; and in the F_1 generation of reciprocal crosses between green and *zonale*, green, variegated, and white offspring were obtained. To explain these results, BAUR suggested as a working hypothesis the possibility that the *zonale* character is inherited through the plastids. The tissue in the *zonale* plant from which the germ cells are formed has only white plastids, and therefore only white progenies are obtained by selfing. The results obtained in the crosses he explained by assuming that some plastids are brought into the fertilized ovum with the pollen nucleus. BAUR points out in his first paper on that subject (2) that his data are not sufficient for a

[3] The variegation described by RENNER (25) in *Oenothera* is considered to be different from that in *Pelargonium*, since it was shown that both nucleus and plastids are instrumental in the transmission of the character.

[4] The writer is much undebted to Professor IKENO for the permission to use his unpublished observations.

final conclusion. He gave his explanation as a working hypothesis for further research. BAUR's explanation, however, has been accepted by the majority of the geneticists who have discussed the matter; and in his later presentations (3) BAUR himself is inclined to give more weight to the hypothesis than was originally intended.

STOMPS (30) suggested a Mendelian explanation for the behavior of *Pelargonium zonale*. He assumed that the character is determined

TABLE IV

BAUR'S DATA ON INHERITANCE OF PELARGONIUM ZONALE

Parents	No. of crosses	F_1 progeny		
		Green	Variegated	White
zonale×zonale	3	....	....	42
white branch×green	1	1	....	....
green×white branch	1	38	7	0
green×zonale	4	139	18	4
zonale×green	4	60	23	0
zonale×white branch	1	....	....	4

by a "perlabile pangene," not going into the details of the explanation. Extensive experiments with *P. zonale* were carried on by NOACK (23, 24, 25), who explains the inheritance of the character as follows:

Als Arbeitshypothese wurde aus diesen Versuchen die Anschauung gewonnen, dass die Buntblättrigkeit der Schecken auf einer Stoffwechselkrankheit beruht, bei der Zellkern und Protoplasma in gleicher Weise beteiligt sein können. So kommt innerhalb der Meristemzellen eine Differenz in den Stoffwechselbeziehungen der beiden durch die Kreuzung vereinigten Partner zustande. Dieser labile Zustand kann durch gegenseitige Anpassung nach der einen oder anderen Seite in einen irreversiblen Zustand überführt werden, und dadurch entstehen rein grüne bzw. rein farblose Keimlinge oder Zweige, oder aber er kann zu einer Art Gleichgewichtszustand zwischen gesundem und krankem Elternanteil führen, und daraus resultieren dann Mantelchimären der verschiedensten Art. Es handelt sich somit bei diesen ganzen Erscheinungen nicht mendelnder Buntblättrigkeit keineswegs um einen Fall echter Vererbung, sondern um eine einfache Übertragung eines mehr oder weniger reversiblen Krankheitszustandes auf die Nachkommen.

In the material used by NOACK, sterility prevented selfing and crosses between *zonale* plants. He succeeded in obtaining only eight seeds from such pollinations, all of which gave white seedlings, con-

firming BAUR'S results. Reciprocal crosses with green, when a *zonale* plant was used as female, gave besides green progenies a larger or smaller number of variegated plants and a few white seedlings; when the green was a female parent very few variegated plants were obtained. In this respect NOACK'S results differed from those obtained by BAUR. Green F_1 plants gave always green progeny when intercrossed, and in the crosses with *zonale* behaved as other green plants. An important contribution of NOACK is the discovery that different *zonale* plants give different proportions of variegated plants in crosses with green, and also that the type of variegation was different in the progeny of the different plants. Certain plants gave a progeny with large proportions of white tissue, while the variegated progeny of other plants had very small white areas. The majority of the variegated seedlings became green in the later stage of development, some of them became white and died, and only a few remained variegated throughout life.

The important facts from BAUR'S and NOACK'S results may be summarized as follows: (1) *Zonale* plants selfed or intercrossed gave white progeny only. (2) In crosses with green plants, white, variegated, and green F_1 seedlings were obtained in varied proportions. According to BAUR, reciprocal crosses gave like results, but in the case of the material used by NOACK a larger proportion of variegated plants was obtained if *zonale* was the female parent. (3) Green F_1 plants, as well as green portions of variegated plants, behaved in F_2 and crosses as normal green. (4) In crosses with green, different *zonale* plants transmitted the variegation in different proportions and intensity.

The data so far obtained do not give sufficient support to either BAUR'S hypothesis or NOACK'S. Results identical with those obtained in the experiments would be expected if it is assumed that the *zonale* character is determined by a dominant gene which becomes mutable (mutating to the gene for green) in crosses with green, or when the plant is heterozygous for green. Such an assumption would be supported by similar behavior observed in several other cases. EMERSON (17) found that the mutability of the gene for variegated pericarp is much increased when the gene is in the heterozygous condition. When maize with very light variegated (almost

white) pericarp was crossed with white pericarp, in the F_1 generation white, variegated, and red seeds were obtained. CORRENS (7, 8) had a similar experience with variegated *Mirabilis*. In the case of the mutable character, miniature wing, found by the author in *Drosophila virilis* (data not yet published), an almost constant miniature becomes highly mutable at the maturation division in the presence of a dominant autosomal factor, and in the presence of another factor becomes very mutable in somatic cells. Reddish, another mutable character known in *Drosophila virilis* (16), mutates only when heterozygous. The fact, observed by NOACK, that reciprocal crosses between *zonale* and green gave different results, could be explained by the assumption that the mutability of the gene for white is increased when brought by the pollen nucleus into other than maternal cytoplasm. Such an assumption is warranted, judging by the results obtained from the studies on mutable genes in *Drosophila* (data not yet published), which indicate that the environment of the gene has an influence on its mutability.

The available data already stated are not adequate to show the validity of the mutable gene hypothesis. The hypothesis, however, is susceptible of some simple tests. If the *zonale* character is determined by a dominant gene for white (W) which becomes mutable when heterozygous (Ww), then the white sectors on the variegated F_1 plants from the crosses *zonale* by green are expected to be Ww. Flowers located on these Ww sectors crossed with pollen from green plants ww are expected to give half of the progeny of the constitution ww, which would be green, and the other half of the constitution Ww, which would be white, variegated, or green. The same flowers in crosses with *zonale* (WW) are expected to have half WW (white) progeny and half Ww (white, variegated, or green); and when selfed one-quarter of the progeny should be WW (white), one half Ww (white, variegated, or green), and one-quarter ww (green). The results from these and similar crosses would be expected to show enough difference to permit a conclusion as to the validity of the hypothesis.

It is well to keep in mind that certain virus diseases produce cholorophyll abnormalities similar in appearance to characters described as being non-Mendelian. Some of the diseases could be trans-

mitted in such a manner that they might easily be mistaken for a heritable character (BLAKESLEE 4). To reduce the possibility of such a mistake to a minimum, thorough inoculation experiments seem obligatory before reaching the conclusion that a character is non-Mendelian.

Summary

1. A chlorophyll variegation is described which is inherited through the female parent only. A variegated plant gives pale green, variegated, and green progeny in varying proportions, irrespective of the kind of pollen used in fertilization.

2. A similar character was already described by ANDERSON. The present one, however, in addition to having an independent origin, differs phenotypically from that previously described.

3. All attempts to transmit the character asexually by inoculation were unsuccessful.

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CHROMOSOME STUDIES IN AESCULUS[1]

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(WITH PLATES III–V)

For several seasons the writer has been studying the pollen formation in *Aesculus*. For the most part, the material for this study has been collected at the Arnold Arboretum and at the Harvard Botanic Garden. The buds were killed in chrom-acetic acid (0.7 per cent), and in Carnoy's fluid, imbedded in nitro-cellulose, and stained in Haidenhain's iron-alum haematoxylin with either safranin or eosin as a counter stain.

The genus *Aesculus* is not a large one, although quite well distributed throughout the Northern Hemisphere. By far the best known species is the common horse-chestnut, *Aesculus hippocastanum* L., which was introduced into the United States about 1746 (1). A microscopic examination of the developing anther shows conditions which are essentially normal. There is slight indication of irregularity in the movement of the chromosomes during meiosis, but it is very rare and not at all pronounced. Moreover, the pollen grains are seldom morphologically imperfect. In other words, from the standpoint of cytology, this form appears to be specific in rank, and is generally considered so by systematists. Hybrids between this species and some red-flowered American buckeye, probably *Aesculus pavia* L. (1), are quite commonly used for ornamentation. Of these *A. rubicunda* Lois. (*A. carnea* Hayne) is perhaps the best known. An examination of the pollen development in this form shows features common to known hybrids. It and the next type (*A. rubicunda* var. *brioti* Carr.) are the only ones studied which show more than twenty chromosomes after reduction. Here we find forty chromosomes as the haploid number, so that the form is tetraploid. During meiosis lagging chromosomes appear frequently. While still held within the mother cell, five or six pollen grains are often present instead of the usual number of four. One of these pollen grains (two,

[1] Contribution from the Department of Biology, Williams College.

if six are present) is much smaller than the rest, and undoubtedly gives rise to the not infrequent small and shriveled pollen grains which are found in the mature anther. In fig. 1 is illustrated the chromosomes arranged at the cell plate during the second (homotypic) division, and one can easily count the forty present. The divisions appear quite regular. In fig. 2 nearly the same stage is shown in another pollen mother cell. The chromosomes in the plate view are not all present, some being cut out of the section, but those in the other spindle, which appears in side view, show a distinct tendency to lag. Figs. 3 and 6 illustrate polyspory as present in the above. Note in fig. 3 the gigantic pollen grain together with four small ones. This condition is not so common as that shown in fig. 6. Doubtless the gigantic grain is just as useless as are the small ones. Fig. 4 illustrates in *A. rubicunda* var. *brioti* how conditions like those shown in fig. 6 are probably developed. Here the second (homotypic) division of the mother cell is illustrated. One of the main spindles shows distinct lagging of chromosomes, but the point of chief interest is the extra spindle. This has doubtless arisen from chromosomes which have dropped off the main spindle in the first (heterotypic) division, and have formed a small spindle of their own. Such a phenomenon has often been noted in cytological work done on other genera (**6, 31, 32, 33, 52**, etc.), and is the usual cause for polyspory. Fig. 8 shows the mother cell of the same variety undergoing the reduction division, and the lagging chromosomes are plainly visible. Fig. 7 illustrates the mature stage of the variety *brioti*, and shows the great difference in size between morphologically good and morphologically sterile pollen grains.

A. hippocastanum var. *baumannii* Schneider is a double flowered variety supposedly of *A. hippocastanum* L. Rehder suggests that the irregularity of chromosome behavior in this form may not be due to hybridization, but to the influence which brings about the doubling of the flowers, and he advocates taking up the whole question as to the cause for the doubling of flowers. Be that as it may, the chromosome irregularities are very suggestive of hybridity. Fig. 9 shows the plate view of two spindles during homotypic division. It will be noted that the haploid number is twenty, as is apparently the case with all species except *A. rubicunda* Lois. and *A. rubicunda*

var. *brioti*. In fig. 10 is shown what is apparently one spindle during the homotypic division. It will be seen that certain chromosomes are small and seemingly double, while others are large and single. This phenomenon cannot be observed in most species, nor do individual chromosomes vary markedly in shape and size as they do in many plants and animals. Fig. 11 is a somewhat later stage during the heterotypic division. Figs. 12, 13, and 15 illustrate the mature pollen grains yet held in the mother cell. In no case was the writer able to observe polyspory, but there seems to be a marked irregularity in the number and size of grains in each mother cell. Another interesting fact is that often whole pollen sacs appear to degenerate, the pollen mother cells becoming vacuolated and empty, and sometimes irregular tapetal-like cells become greatly enlarged and fill the whole sac.

A. glabra Willd., a common tree of the Mississippi Valley, appears perfectly normal in its meiosis, and hence is a good species from the cytological point of view. Two of its so-called varieties, however, proved interesting. *A. glabra* var. *buckleyi* Sarg., a form found growing in certain localities in Missouri, Iowa, and Kansas, shows a very large proportion of sterile pollen grains. Unfortunately the writer was unable to procure any of the early stages of development, and so cannot report upon the chromosome behavior. *A. glabra* var. *leucodermis* Sarg. is a tree reaching 18–20 m. in height, and is characterized by having five-foliolate leaves, yellow flowers, and pale smooth bark. An examination of the chromosomes during meiosis showed distinct lagging during the heterotypic division, and slight irregularities during the homotypic division. In the mature stage a small percentage of morphologically sterile pollen grains appears.

A. arguta Buckley is considered by systematists a closely related species to *A. glabra* Willd. It is a "small narrow shrub tree-like in habit," found growing naturally in Oklahoma and a few localities in northern and central Texas. Here we find distinct lagging of chromosomes in the first division, as shown in fig. 28. The chromosomes are more normal in the homotypic division, and yet there is also some irregularity. In the mature stage there is a large percentage of morphologically sterile pollen.

A. octandra Marsh (Sweet buckeye), also called *A. flava* Ait., grows as a large tree or shrub in rich woods from western Pennsylvania northward to Wisconsin, westward to Iowa, and southward to Georgia, Alabama, and Texas. Its pollen development shows no irregularity to indicate that the plant is not of specific rank. One tree, however, labeled *A. octandra* and growing in the Harvard Botanic Garden, gave strong evidence of being a hybrid. There was a large number of sterile pollen grains at maturity, and lagging of chromosomes was plainly evident in the reduction division. Fig. 23 shows the situation during heterotypic division, when the majority of chromosomes have arrived at the plate preparatory to dividing. The lagging of chromosomes is quite evident. In fig. 26 a later view is shown, after the majority of the chromosomes have divided and passed to the poles. It will be seen that one pair of chromosomes is distinctly behind the rest, and that still another chromosome is slow in joining with the general group.

One form, known as *A. octandra* var. *hybrida* (DC) Sarg. and characterized by having purple or red flowers, is found growing along the Alleghany Mountains from West Virginia southward and westward. This is considered by REHDER as a hybrid between *A. octandra* Marsh. and *A. pavia* L. Sections of this form show one of the best examples observed of polyspory. In fig. 20 such a pollen mother cell is illustrated. It will be seen that there are seven pollen grains of varying sizes visible. Lagging is also in evidence during the heterotypic and homotypic divisions. Fig. 21 illustrates such lagging in the homotypic division. Only one division is shown, since the other was largely eliminated from the section by the microtome knife. Because the writer was unable to obtain any mature stages, the situation with regard to morphologically sterile pollen could not be studied. However, one would expect to find the sterile grains abundant.

As already stated, *A. flava* Ait. is considered by systematists a synonym for *A. octandra* Marsh. The *A. flava* studied in this article was collected at the Harvard Botanic Garden, and from the standpoint of the pollen formation certainly behaves like a hybrid. Fig. 24 illustrates lagging during the heterotypic division, when the majority of chromosomes have arrived at the cell plate. Fig. 27 shows a stage similar to fig. 24, but during the second division.

Lagging of chromosomes may clearly be observed. In fig. 18 the mature pollen grains appear. These are largely degenerated and nearly empty of protoplasm.

The form *A. discolor* Pursh was apparently first discovered in Lyon, Georgia. As first described, it is a shrub not more than about four feet high with "leaflets tomentose on lower surface and flowers yellow, white and purple, variegated." The description has caused much confusion. In 1824 De Candolle referred it to his *A. hybrida*, believing it to be a hybrid between *A. lutea* (*octandra*) and *A. pavia* L. In 1828 Torrey and Gray made a variety *discolor* of *A. pavia* L., to which they doubtfully referred the plants of Pursh, Lindley, and De Candolle. Later Gray made this plant a variety *purpurascens* of *A. flava* Ait. Sargent believes *A. discolor* Pursh to be a true species (45). One variety, *A. discolor* var. *mollis* n. var., found growing in Georgia and Alabama, has bright scarlet flowers. In fig. 25 is illustrated the heterotypic division in this variety, and it will be seen that lagging is in evidence. In the mature stage considerable morphological sterility of pollen is also present.

Another form, *A. octandra* var. *discolor* Rehder, very closely related if not identical with *A. discolor* Pursh, also shows lagging chromosomes during the heterotypic and homotypic divisions, and many sterile pollen grains are present at maturity. Fig. 22 gives evidence of the lagging during heterotypic division. It will be noted that one pair of chromosomes is especially slow in reaching the plate.

A. harbisonii Sarg. is a late flowering variety considered to be a hybrid between *A. discolor* var. *mollis* n. var. and *A. georgiana* Sarg. It is a rare shrub with "rose-colored calyx and canary yellow petals tinged with rose toward the margin." Further proof for its hybrid origin appears when a cytological study of its pollen formation is carried out. It shows excellent examples of lagging during the heterotypic division, and less commonly during the homotypic division. In fig. 30 the heterotypic division is portrayed, and it will be seen that a large number of chromosomes are slow in reaching the plate of the spindle. In fig. 29 the homotypic division is illustrated, and one pair of chromosomes will be observed to be slow in arriving at the equator of the spindle. The mature pollen sac does not show a large amount of morphologically sterile grains, but there are always some present.

A. georgiana Sarg. was first discovered near Stone Mountain in Georgia, but has since been found to range into the Piedmont region of North Carolina, where it becomes a small tree. It has been given specific rank by SARGENT. From the cytological standpoint it appears to be of hybrid origin. In fig. 19 the chromosomes are shown well arranged for counting, and it will be observed that there are twenty. In fig. 17 it will be seen that there is plenty of lagging during the heterotypic division. In fig. 16 five pollen grains are shown held within their mother cell. One grain distinctly smaller than the rest lies directly over a larger one. At maturity a large proportion of morphologically sterile pollen grains are present.

In fig. 5 the heterotypic division of *A. mutabilis* var. *induta* n. hyb. Sarg. is shown. This form is considered a hybrid by the systematists, and it will be seen that cytological evidence during the heterotypic division bears out this viewpoint. Lagging chromosomes are also present to some extent during homotypic division, although not so common. *A. mutabilis* var. *pendulifolia* Sarg. is also considered to be a hybrid. Its parents are thought to be *A. discolor* var. *mollis* n. var. and *A. neglecta* Sarg., also considered to be a hybrid. Lagging of chromosomes is abundant in this form during heretotypic division, and many sterile pollen grains appear at maturity.

A. woerlitzensis Koehne, E. is a form of unknown origin which came first from Europe. Cytologically it appears to be a hybrid, showing plentiful lagging of chromosomes in the heterotypic division and a large proportion of sterile pollen at maturity.

Discussion

The literature on meiosis in plants makes clear that much study has been given the subject, and that many different points of view have arisen. Every student of botany is acquainted with normal meiosis, at least in the vascular plants, and knows that all research on the subject has furnished increasing proof that each species of plant, under normal conditions, has a constant number of chromosomes, and that species closely allied to each other tend to have the same, or nearly related chromosome counts.

In the majority of cases meiosis is perfectly regular. It is not with the normal condition that the great interest lies, however, but with certain irregularities which are discussed in this article.

We usually find in the life history of a vascular plant a continuous cycle of alternation between the sexual (haploid) and non-sexual (diploid) phases of the individual. There is now an increasing list of examples where this is not the case, and where by one of a variety of ways the alternation is broken. Such phenomena are listed under the heading of apomixis, and, although not noted in *Aesculus*, are known to be often the result of hybridization.

Another extremely interesting variation, and one which at the present time is occupying the attention of cytologists, is the occurrence of an increased number of chromosomes. Whereas in most plants each genus has a definite gametophytic (haploid) number of chromosomes in the reproductive cells, examples are increasing where certain species of the same genus may have instead the number increased sometimes as high as ten times the original. These polyploid forms, in which the number of chromosomes is some multiple of the regular haploid number common to the genus in question, are often spoken of as euploid (52). On the other hand, certain genera, as for example *Carex* (14), show among their various forms chromosome numbers which are greater than the haploid number but which are not a multiple thereof. Such a condition is known as aneuploid. JEFFREY (21) has suggested the terms artiploid, perissoploid, and dysploid instead of the preceding, for the forms respectively where the chromosome numbers are even (diploid, etc.), uneven (triploid, etc.), and where the number does not come under the law of multiples (*Carex*, *Crepis*, etc.).

Along with polyploidy, although frequently also where the chromosome number is not increased, one often finds that certain chromosomes lag behind during meiosis, and either eventually catch up with the others to enter into the structure of the daughter nuclei, or become cast off into the surrounding cytoplasm. In many cases these laggards appear to be univalent and hence slower in their movements, but in certain cases the univalents appear to move faster than the bivalents, and consequently there seems to be no fixed rule.

When chromosomes lag behind and are extruded into the cytoplasm, they may degenerate or they may form spindles of their own during the second meiotic division, and develop small excess nuclei. These nuclei may degenerate or may become the center of small

pollen grains, which, added to the usual number of four, cause an increase in the number in each mother cell. Such small grains usually do not reach maturity but become shriveled and empty (polyspory).

ERNST (9), ROSENBERG (41), WINGE (57), TÄCKHOLM (52), and others believe hybridization to be one of the most common, although not the only cause for sterility in plants and animals. Many artificial crosses have been carried out and much morphological sterility has been observed. Along with this sterility apomixis and lagging chromosomes, as well as polyploidy and polyspory, may occur. In the now classic *Drosera* cross of ROSENBERG (39) it is definitely shown that when these two species of different chromosome numbers (*D. longifolia* with twenty in the haploid and *D. rotundifolia* with ten in the haploid) are crossed, the resulting hybrid shows ten bivalent and ten univalent chromosomes. The latter lag behind the former, especially in the heterotypic division. The lagging chromosomes are often extruded into the cytoplasm, and form small spindles from which extra pollen grains are developed.

Since this investigation many similar ones have been made. In his investigation with *Oenothera* crosses, GATES (11, 12, 13, etc.) has found many aberrations similar to those found in *Drosera*, and suggests that "irregular tetrad-formation puts under suspicion the purity of any plant in which it is found." DIGBY (8), working with *Primula* hybrids, was able to obtain tetraploid offspring. YASUI (59) and LJUNGDAHL (30), studying *Papaver* hybrids, found very interesting irregularities. TISCHLER (54, 55), in his results with *Ribes* and *Bryonia* hybrids, observed such irregularities as lagging chromosomes, polyploidy, and polyspory. Likewise in the work of SAKAMURA (44), and more recently of KIHARA (26, 27, 28, 29) and of SAX (46, 47, 48) with *Triticum* hybrids, these irregularities also often appear.

Several years ago JEFFREY (20) pointed out that there are many natural hybrids in existence which, while propagating themselves and breeding more or less true, are nevertheless hybrids in origin (crypthybrids). Many of these are known to propagate themselves either by purely vegetative means or through apomixis, but there are others which are forming enough viable seeds to greatly increase their number. One at once wonders why such plants which should

be heterozygous do not segregate out. Certain geneticists have attempted to answer the point by supposing the presence of so-called "balanced lethal" factors which make the plant appear as a heterozygote. Whether this explanation is the right one or not seems hard to determine, but in any case such hybrids have long given the taxonomist great trouble in determining the proper classification of the plants in question.

Recently much study has been given these natural hybrids, and very interesting results have been obtained. The Rosaceae have long been known to have several genera which show great irregularities. In 1916 Miss COLE (7) showed through a study of various species of *Rosa* that much sterility of pollen is present. The studies of TÄCKHOLM (52), BLACKBURN and HARRISON (6), and PENLAND (37) have revealed much about the cause for this condition. They find great irregularities, such as polyploidy, polyspory, lagging chromosomes, and apomictic phenomena. The writer (15) showed the presence in *Rubus* of much morphological sterility of pollen. LONGLEY (32) cleared up the situation and showed meiotic peculiarities closely resembling those recorded for *Rosa*. Miss STANDISH (51) reported much sterility in pollen in the very unsettled genus *Crataegus*, and later LONGLEY (33) also showed the situation here to be similar to that for *Rosa* and *Rubus*. Among the Compositae, TAHARA (53) and ISHIKAWA (18) for *Chrysanthemum*, ROSENBERG (41) for *Hieracium*, ROSENBERG and others (42) for *Crepis*, and HOLMGREN (16, 17) for *Erigeron* and *Eupatorium*, have found similar peculiarities. The work of HEILBORN (14) with *Carex* is extremely interesting. In *Carex*, as already mentioned, there is a wide range of chromosome numbers, forming a large series of aneuploid types, and together with this polyploidy occur other irregularities so commonly found in hybrids. Finally, JEFFREY and HICKS (23, 24) have recorded an apparent hybrid form of *Isoetes*, found growing in Cape Breton Island, which shows two types of chromosomes (large and small). The small appear constantly to lag behind the large. This form also shows an extreme sterility. The authors also report a very abundant lagging of chromosomes in the Boston fern. This is known to be a very sterile form propagating by vegetative methods. If we accept the hybrid origin of this fern as fact, and it seems hard not to when

one takes into consideration how it originated as well as its chromosomal irregularities, it is easy to understand why so many clones appear, as reported by BENEDICT (3, 4, 5).

In the preceding paragraphs an attempt has been made to show that the irregularities recorded in certain plant genera are common to both artificial and natural hybrids; therefore when such irregularities, or some of them, appear in certain forms of *Aesculus* they put the purity of their ancestry under suspicion.

Conclusions

Aesculus gives abundant proof, in addition to that already obtained through work done upon *Rosa*, *Crataegus*, *Rubus*, *Oenothera*, and other plants, that chromosome irregularities and morphological sterility of pollen are usually present in known hybrids, and thus, when found in plants of uncertain origin, are indicative of hybrid ancestry.

Since the chromosome count in most forms of *Aesculus* shows twenty chromosomes present in the reduced stage, it would not be expected that the pollen formation would present as many irregularities as in a genus like *Rubus*, where polyploidy has been found as high as the octoploid number. However, chromosome irregularities and morphologically sterile pollen are abundant in *Aesculus*.

A study of the pollen formation in *A. hippocastanum*, *A. glabra*, and *A. octandra* gives additional evidence that these forms should be considered of specific rank. Likewise plants such as *A. rubicunda*, *A. rubicunda* var. *brioti*, *A. octandra* var. *hybrida*, *A. harbisonii*, *A. mutabilis* var. *induta*, and *A. mutabilis* var. *pendulifolia*, which are considered hybrids by systematists, appear to be such when the pollen formation is taken into consideration. *A. woerlitzensis*, a form whose origin seems to be uncertain, is apparently a hybrid.

When the pollen formation of the other varieties of *Aesculus* is considered, the results appear to be more or less at variance with the opinion of the systematists. *A. hippocastanum* var. *baumannii*, considered by the systematists a variety of *A. hippocastanum*, shows marked irregularity in pollen formation. *A. glabra* var. *buckleyi* and *A. glabra* var. *leucodermis*, considered varieties of *A. glabra*, show many of the irregularities of hybrids. *A. arguta*, considered as a

species closely allied to *A. glabra*, has plentiful lagging of chromosomes and an abundance of morphologically sterile pollen at maturity. *A. flava*, considered as another name for *A. octandra*, at least in the material studied, shows plenty of evidence for a hybrid origin. The variety *mollis* of the supposed species *A. discolor* has lagging chromosomes and morphologically sterile pollen. *A. octandra* var. *discolor*, a form possibly identical with *A. discolor*, in chromosome behavior and in sterility of pollen appears to be of hybrid origin. Finally, *A. georgiana*, named by SARGENT and considered by him of specific rank, not only shows lagging chromosomes and sterile pollen, but also polyspory.

A. rubicunda and *A. rubicunda* var. *brioti* are of special interest, since their chromosome count after the heterotypic division is forty; hence they must be ranked as tetraploid.

The writer wishes to express his sincere gratitude to C. S. SARGENT, ALFRED REHDER, E. C. JEFFREY, and others for their aid in collecting the material used in this work. Many of the buds were gathered by the late JAMES AUSTIN.

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EXPLANATION OF PLATES III–V

Magnification of figures 1400 unless otherwise specified.

FIG. 1.—*A. rubicunda* Lois.: section of pollen mother cell during homotypic division, showing chromosome count of forty.

FIG. 2.—*A. rubicunda* Lois.: section of pollen mother cell during homotypic division, showing lagging of chromosomes.

FIG. 3.—*A. rubicunda* Lois.: mature pollen mother cell, showing irregularity in size of pollen grains; one grain gigantic in size and others small and nearly empty.

FIG. 4.—*A. rubicunda* var. *brioti* Carr.: section of pollen mother cell during homotypic division, showing lagging of chromosomes in one spindle and small third spindle formed from chromosomes dropped from spindle during heterotypic division.

FIG. 5.—*A. mutabilis* var. *induta* n. hyb. Sarg.: section of pollen mother cell during heterotypic division, showing lagging of chromosomes.

FIG. 6.—*A. rubicunda* Lois.: mature pollen mother cell showing polyspory; four of pollen grains normal in size while other two are dwarfed.

FIG. 7.—*A. rubicunda* var. *brioti* Carr.: mature pollen grains showing empty grains mingled with those normal in appearance; $\times 700$.

FIG. 8.—*A. rubicunda* var. *brioti* Carr.: pollen mother cell during heterotypic division, showing lagging of chromosomes in spindle.

FIG. 9.—*A. hippocastanum* var. *baumannii* Schneider: pollen mother cell during homotypic division, showing chromosome count of twenty.

FIG. 10.—*A. hippocastanum* var. *baumannii* Schneider: pollen mother cell during homotypic division (only one spindle), illustrating lagging of chromosomes (note that some chromosomes appear paired).

FIG. 11.—*A. hippocastanum* var. *baumannii* Schneider: pollen mother cell during heterotypic division showing chromosomes arriving at equator of spindle with some lagging behind.

FIGS. 12, 13, 15.—*A. hippocastanum* var. *baumannii* Schneider: pollen mother cells with pollen grains already formed, showing irregularity in number and size of grains.

FIG. 14.—*A. hippocastanum* var. *baumannii* Schneider: homotypic divisions of pollen mother cell showing marked lagging of chromosomes in both spindles.

FIG. 16.—*A. georgiana* Sarg.: section of pollen mother cell showing polyspory.

FIG. 17.—*A. georgiana* Sarg.: section of pollen mother cell during heterotypic division showing lagging of chromosomes.

FIG. 18.—*A. flava* Ait.: mature pollen grains showing contrast between morphologically sterile and perfect pollen grains; $\times 700$.

FIG. 19.—*A. georgiana* Sarg.: section of pollen mother cell during heterotypic division, showing chromosome count of twenty.

FIG. 20.—*A. octandra* var. *hybrida* (DC) Sarg.: section of pollen mother cell showing polyspory.

FIG. 21.—*A. octandra* var. *hybrida* (DC) Sarg.: section of pollen mother cell during heterotypic division, showing lagging chromosomes.

FIG. 22.—*A. octandra* var. *discolor* Rehder: section of pollen mother cell during heterotypic division showing lagging of chromosomes.

FIG. 23.—*A. octandra* Marsh. (?): section of pollen mother cell during heterotypic division showing lagging of chromosomes.

FIG. 24.—*A. flava* Ait.: section of pollen mother cell during heterotypic division showing lagging of chromosomes.

FIG. 25.—*A. discolor* var. *mollis* n. var.: section of pollen mother cell during heterotypic division showing lagging of chromosomes.

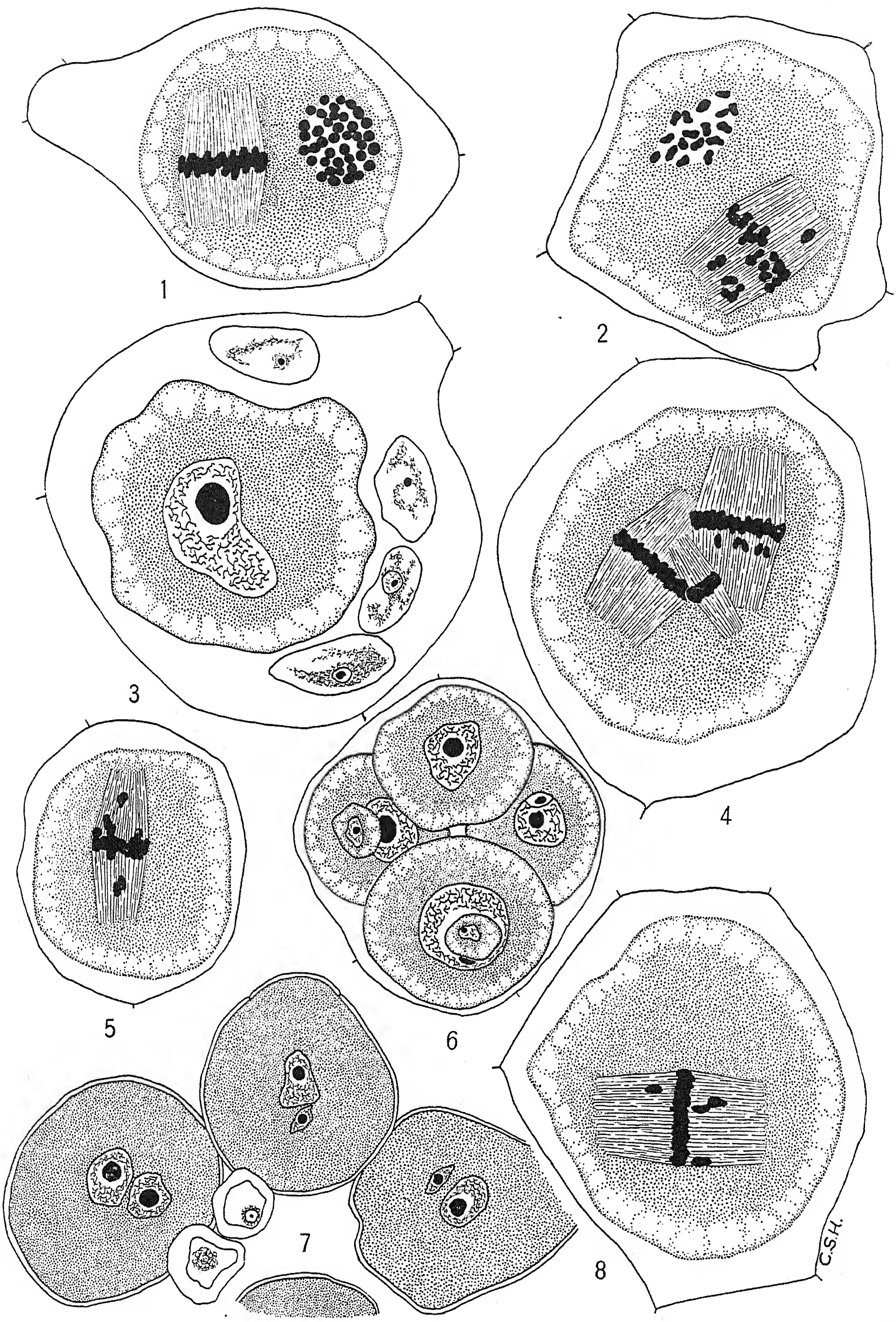
FIG. 26.—*A. octandra* Marsh. (?): section of pollen mother cell during heterotypic division (later stage than shown in fig. 23), showing distinct lagging of one pair of chromosomes.

FIG. 27.—*A. flava* Ait.: section of pollen mother cell during homotypic division showing lagging chromosomes.

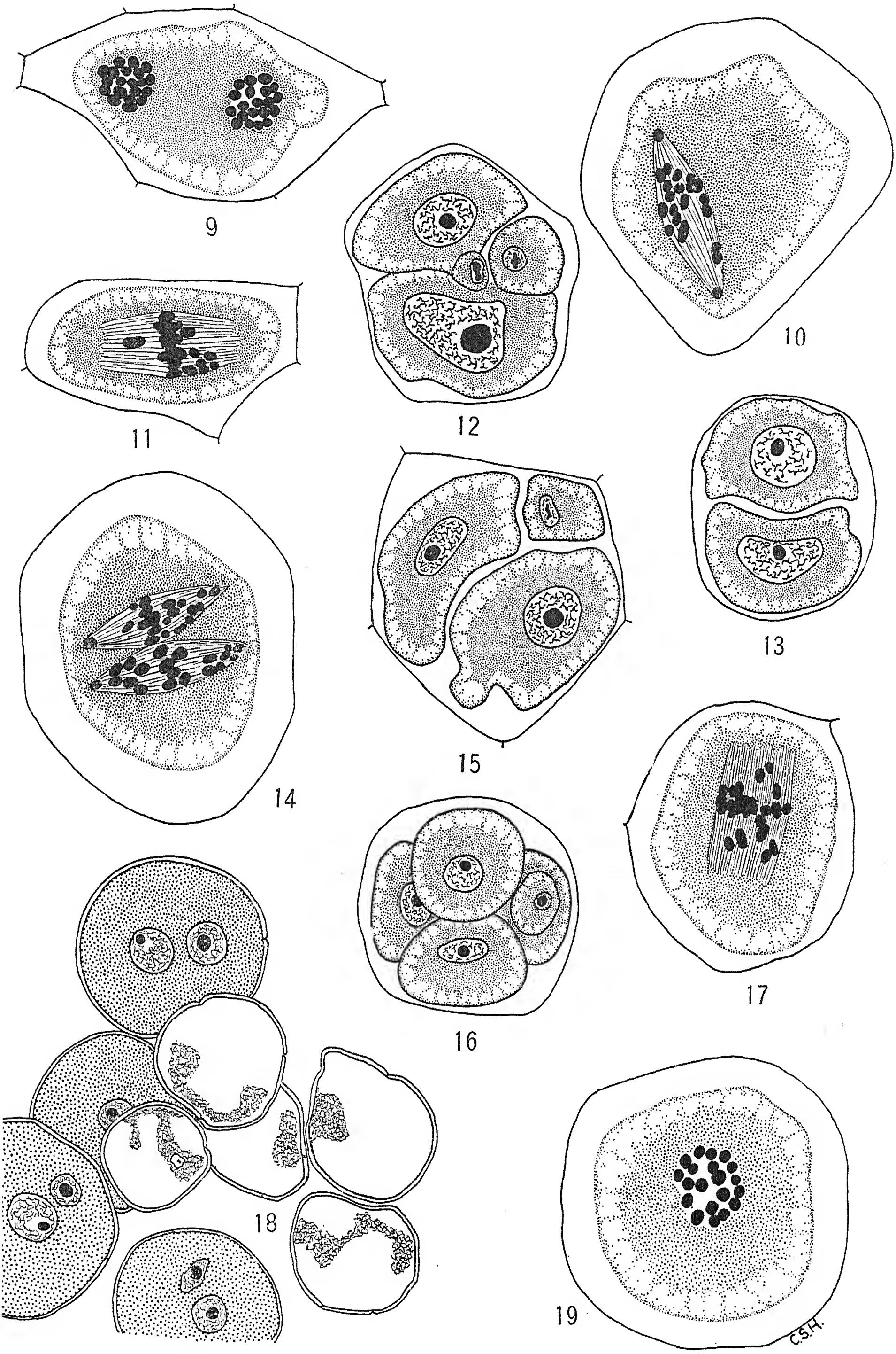
FIG. 28.—*A. arguta* Buckley: section of pollen mother cell during heterotypic division showing lagging of chromosomes.

FIG. 29.—*A. harbisonii* Sarg.: section of pollen mother cell during homotypic division showing lagging of chromosomes.

FIG. 30.—*A. harbisonii* Sarg.: section of pollen mother cell during heterotypic division showing lagging of chromosomes.



HOAR on AESCULUS



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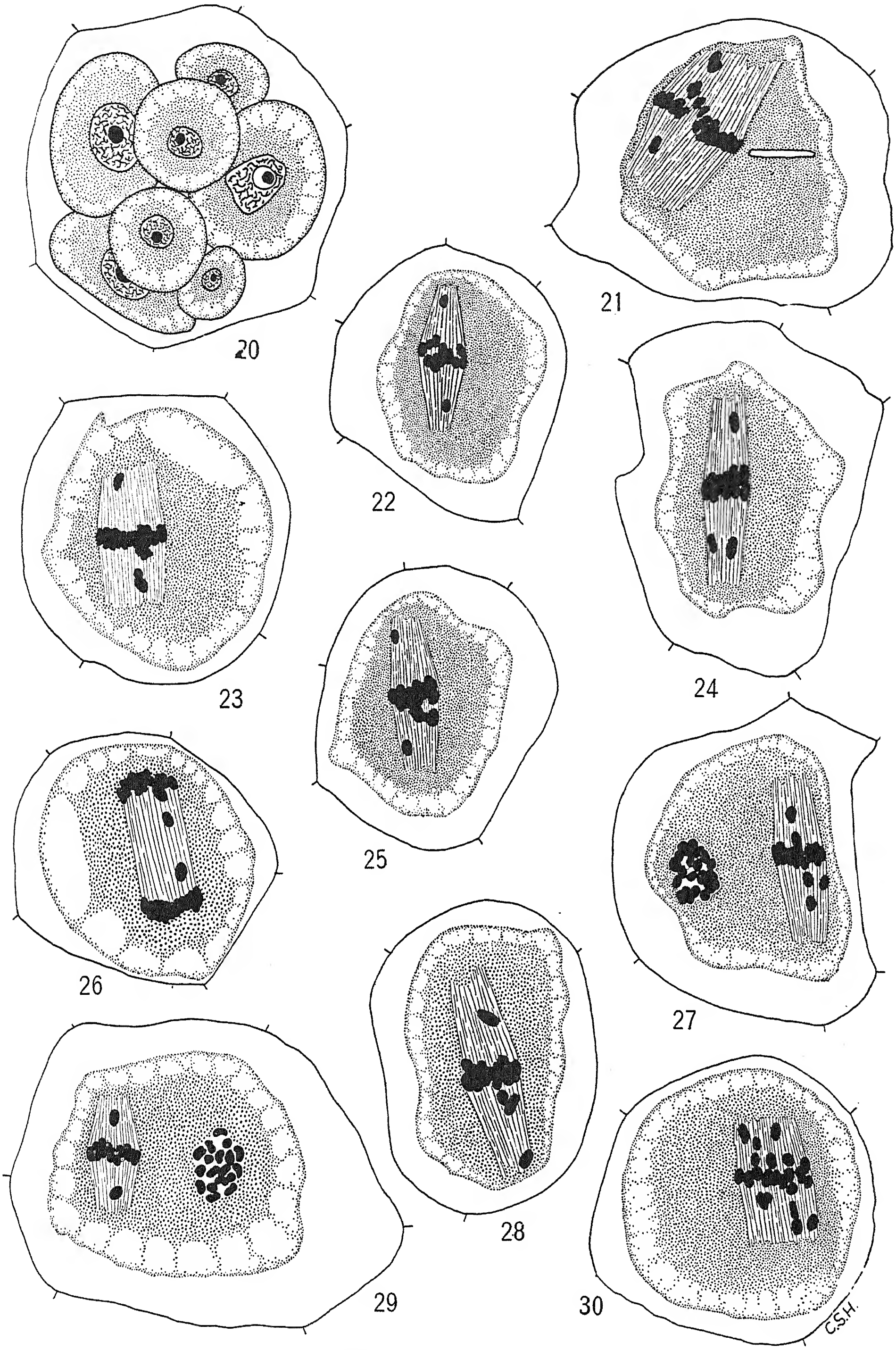


HOAR on AESCULUS

CYTOLOGICAL STUDIES IN THE GENUS WISTERIA[1]

MURIEL V. ROSCOE

(WITH PLATE VI AND SIX FIGURES)

Frequently vines of Asiatic *Wisteria* in cultivation are entirely given over to vegetative development and never produce flowers or fruit. Both the Asiatic and American species present varying degrees of pollen sterility. This study was undertaken to see whether any morphological basis for these conditions could be found.

Materials and methods

The collection of *Wisteria* at the Arnold Arboretum contains most of the varieties of the Asiatic and American species. The buds used were obtained chiefly from this source. Additional material of *W. sinensis* was obtained from the Botanic Gardens of Harvard University, as well as from privately owned vines. Collecting was confined to warm days and was carried on only in the middle of the day.

The buds were cut with a sharp razor and put immediately into the fixing fluid. Both chromo-acetic (0.75 per cent) and Carnoy's solutions were tried as fixatives, and the results were found to be superior after the Carnoy's. The air was exhausted by means of an air-pump. After fixing and subsequent washing, the buds were softened and bleached in a 10 per cent solution of a saturated solution of sodium chlorate in hydrofluoric acid. Then after thorough washing and gradual dehydration in alcohol, they were imbedded in nitrocellulose. Extensive use was made of the mass method for nitrocellulose, originated by JEFFREY (20). The sections were cut at thicknesses of 5 and 10 μ, and stained in Haidenhain's iron-alum haematoxylin. For pollen study, safranin was used as a counter stain.

The sections were studied with the assistance of a 1.5 mm. Zeiss apochromatic objective and nos. 5 and 12 compensating oculars. All

[1] Contribution from the Laboratories of Plant Morphology, Harvard University.

text figure drawings were made with the aid of a Spencer camera lucida, used in conjunction with the 1.5 objective and 12 ocular, and were reduced one-half in photographing. The figures of the plate are free-hand drawings, but are accurate representations and give magnifications of approximately 1500.

The nomenclature used for the species and varieties obtained from the Arnold Arboretum is in accordance with the labeling of the plants there, while I am indebted to Professor C. S. SARGENT for furnishing the authorities for these names. For the identification of *W. sinensis* gathered from other sources, use has been made of BAILEY's manual (1).

Observations

ASIATIC SPECIES

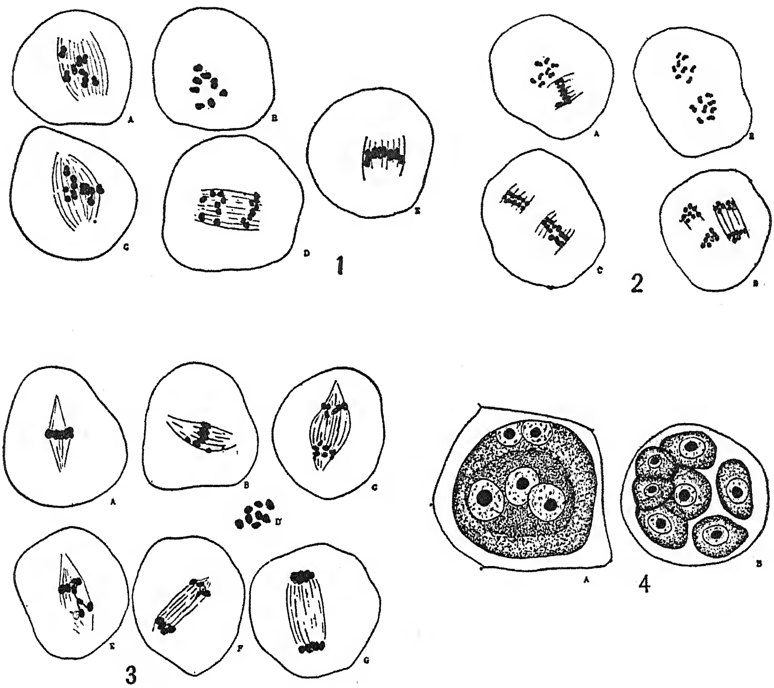
W. floribunda DC., var. *macrobotrys* Rehder & Wilson, $\times = 8$.—All phases of both the heterotypic and homotypic divisions manifest regularity. The tetrads formed show no suggestion of polycary or polyspory, yet the mature pollen rarely exhibits more than 75 per cent of perfect grains.

W. floribunda DC., var. *alba* Rehder & Wilson, $\times = 8$.—The behavior of this variety showed a deviation from the regularity noted for var. *macrobotrys*, particularly in regard to the metaphase of the first division. Fig. 1 *A* and *C* may be considered representative of the condition which obtains if the chromosomes are "caught" on their way to the plate, and the relation of the chromosomes to the spindles is comparable with that shown in hybrid meioses. Despite a lack of concerted action in heterotypic anaphase (fig. 1 *D*), apparently all the chromosomes become included in the daughter nuclei. In the homotypic division (fig. 2 *A–D*) the abnormalities are negligible, and the tetraspores are formed in the usual fashion. However, mature pollen sacs disclose only about 45–50 per cent of protoplasmic pollen grains.

W. floribunda DC., var. *rosea* Rehder & Wilson, $\times = 8$.—The material was not sufficient to furnish a clear idea of the progress of the divisions. Unimbedded material showed 75–85 per cent of the pollen in young buds to be fully protoplasmic.

W. sinensis Sweet, $\times = 8$.—Fig. 7 shows diakinesis with 8 bivalent chromosomes, a highly vacuolated nucleolus, and a fine, loose

network of threads. The chromosomes show some tardiness in forming the metaphase plate, but usually they all arrive there before the beginning of anaphase. The separation of the halves of the bivalents, and a transition to anaphase are indicated in fig. 8. Interkinesis is of some duration and is featured by abundant nucleoli, as



FIGS. 1–4.—Fig. 1, *W. floribunda* var. *alba*, heterotypic divisions: *A*, early metaphase; *B*, metaphase (polar view); *C*, early metaphase; *D*, anaphase; *E*, metaphase; fig. 2, *W. floribunda* var. *alba*, homotypic divisions: *A*, *B*, metaphase; *C*, *D*, anaphase; fig. 3, *W. sinensis*, meiotic abnormalities occurring in pollen mother cells of midsummer buds: *A*, *B*, *D*, metaphase; *C*, *E*, *F*, anaphase; *G*, telophase; fig. 4, *W. venusta:* *A*, polycary; *B*, polyspory showing 7 microspores formed from one pollen mother cell.

many as 4 (fig. 11) being frequently observed. The meaning of the conspicuous masses of black-staining material in the cytoplasm is vague. Tetraspores resulting from the homotypic division all show complete normality in their early stages (fig. 13), and degeneration occurs only later.

The pollen grains often remain in masses, and frequently are unable to form an exine coat. In other cases the grains develop walls, but become crumpled and lose their protoplasmic contents. At most, but 60–65 per cent of viable grains mature (fig. 5).

Fig. 3 shows the irregularities observed in buds of the midsummer flowers of *W. sinensis*. Fig. 3 *B* illustrates a frequent condition; *C*, *E*, and *F* picture various anaphases characterized by abnormality with chromatin spread along the spindle fibers. In some cases such chromatin actually bridges the gaps between chromosomes (*E*, *F*). Telophases of a normal character are occasionally achieved (*G*). In these divisions abnormality is very much the rule, but in spite of this at least a few good pollen grains are formed, and some of the late flowers frequently set seed. Although the figures seen in the spring flowers may scarcely be called irregular, neat plates are rarely attained in *W. sinensis*, and in the summer flowers the abnormalities of meiosis are very conspicuous.

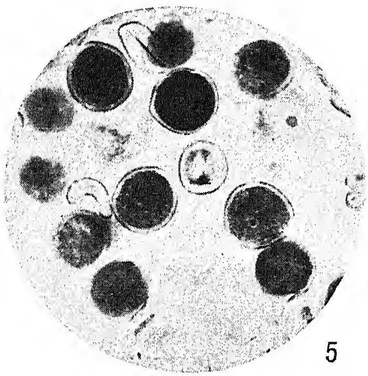
W. venusta Rehder & Wilson, $\times = 8$.—Heterotypic stages differ little from those recorded for *W. sinensis*. The amount of material containing homotypic telophases was small, but in this, one decided case of polycary with five nuclei was found (fig. 4 *A*). A large number of locules revealed microspores in the tetrad stage and many cases of polyspory. As many as 7 spores were observed as resulting from a single pollen mother cell (fig. 4 *B*). Until a study of more material of homotypic divisions showing distribution of the chromosomes is possible, no conclusions are warranted in regard to the appearance of polycary and polyspory in this species. The pollen shows a very high percentage of sterile grains, of which many are conspicuously small. Probably very few are capable of effecting fertilization.

American species

W. macrostachya Nutt., $\times = 8$.—The material did not disclose sufficient division figures to allow of any conclusions. The microspores appear to be formed in tetrads, no cases of polyspory having been observed. In a few cases the tetraspores seemed unable to separate from one another. Cytomixis takes place frequently, and has been seen to occur between daughter tetraspores of different pollen mother cells. The pollen contains almost no good grains.

W. frutescens (L.) Poir., var *alba* Rehder & Wilson, $\times=8$.—This proved to be the only variety of the American species containing satisfactory stages for detailed study. The figures disclose nothing very different from the chromosome relations expressed in the Asiatic species. In diakinesis the chromosomes are clearly bivalents, and the heterotypic division apparently proceeds normally.

Although the material containing the homotypic stages was collected only in chromo-acetic, and consequently showed the chromo-



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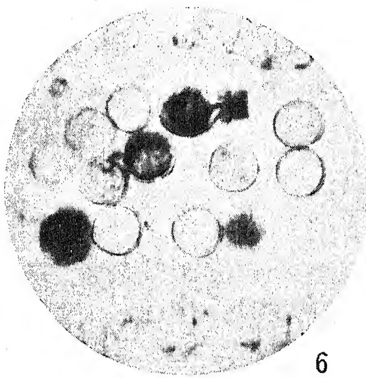

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FIGS. 5–6.—Fig. 5, *A*, pollen of *W. sinensis;* c. 600×; fig. 6, pollen of *W. frutescens* var. *alba;* c. 600×.

somes massed together at the plates, there was no indication of anything but regularity of chromosome distribution. In those tetrads examined, no case of polycary or polyspory presented itself. The pollen proved very sterile, only a few grains in a locule appearing normally protoplasmic (fig. 6).

Discussion

FIXATIVES.—Buds fixed in Carnoy's fluid and in chromo-acetic solution gave very different results. Sections containing the pollen mother cells show more shrinkage after Carnoy fixation than do those preserved in chromo-acetic. In contrast with chromo-acetic treatment, following Carnoy's the chromosomes show no tendency to clump; consequently ideal sections for counting are obtained. Thus whenever the Carnoy material provided the desired stages, this

alone was studied, and chromo-acetic-fixed material was resorted to only when absolutely necessary.

An interesting comparison of the two fixatives is in their effect on the perinuclear zone. This zone has been observed to be omnipresent in *Wisteria* cells during division, and may be found also in the young tetrads. Its significance is not at all apparent, although it seems similar to the "perikaryoplasm" in *Cobaea scandens* described by LAWSON (26), and to such zones as occur in cotton (CANNON 6) and the Cucurbitaceae (CASTETTER 8). The nature of the zone seems to have been modified by the fixative used. After Carnoy's, this zone is most definite in extent and is strongly contrasted externally with the cytoplasm and internally with the clear nuclearplasm, while after chromo-acetic its outer and inner limits are not sharply set off, and often it is found to have invaded the spindle. DEVISÉ's observations (11) on *Larix* led him to conclude that the felted perinuclear zone is an artifact produced by the fixing fluids. Those fluids containing large amounts of acetic acid modified that part of the cytoplasm surrounding the nucleus with its contained particles to give a felted appearance.

Black-staining masses are of frequent occurrence in the cytoplasm in all the *Wisteria* preparations, but they are more conspicuous in those fixed in chromo-acetic. For a study of the chromosomes and their relationships, Carnoy's proved to be a suitable fixative.

NUCLEOLI.—WILSON (40) discusses nucleoli as being of two sorts, plasmosomes and karyosomes, and considers their origin "still to a considerable extent in doubt; but the evidence is accumulating that all forms of them may be directly derived from the chromosomes."

The nucleolus seen in prophase of *Wisteria* divisions takes a very dark haematoxylin stain, but just previous to the dissolution of the nuclear membrane at the close of diakinesis, it becomes highly vacuolated and reacts less strongly to haematoxylin (fig. 7). The appearance suggests that there is a relation between the nucleolus and chromatin.

After the first division, two, three, or more nucleoli appear, and frequently are formed before the chromosomes have lost their morphological individuality, and thus before the nucleus can be said

to have become organized for the resting period. The correlation between chromatin and nucleoli is not as clear as in the prophase.

CYTOMIXIS.—In *Wisteria macrostachya*, cytoplasmic strands were seen connecting pollen mother cells at various stages of development, chiefly at synizesis but also even between formed tetraspores. The presence of this phenomenon at so late a stage is rare in plants, for mention of it in the literature is usually confined to synapsis stages.

HETEROTYPIC AND HOMOTYPIC DIVISIONS.—The roses are divided by TÄCKHOLM (37) into three major groups: (1) In which normally only paired chromosomes appear during the reduction division. Here are contained diploid, tetraploid, hexaploid, and octaploid species and hybrids. (2) In which both paired and unpaired chromosomes appear, but in multiples of seven. The Caninae constitute this division. (3) In which the chromosomes are not in multiples of seven. Forms with such a constitution are termed aneuploid hybrids, and originated by the hybridizing of Canina roses. The first group contains diploid species and hybrids, which are the forms of interest in relation to *Wisteria*.

TÄCKHOLM considers the diploid hybrids are either long-cultivated hybrids with uncertain histories, or forms which have arisen spontaneously, or the products of known crossings. A strong affinity exists between the chromosomes of such hybrids, and there is little disturbance in the divisions of the pollen mother cells. This investigator found the course of meiosis regular, and the tetrads and even the young pollen grains looked usually entirely normal. The mature grains were not studied.

Of the known crosses, *R. chinensis*×*multiflora*, the "Dawson rose," shows no deviations from the normal in meiosis, and the young microspores appear of similar size and health after they have rounded off. Similarly, in *R. microphylla*×*rugosa* only occasionally is the affinity weakened in one of the seven pairs, and metaphase, anaphase, and telophase usually show no disturbance; only in a few cells among hundreds is a separate chromosome found lying free in the plasma.

A very comparable situation is found in *Wisteria*, where the history is also one of long cultivated forms which quite possibly have

arisen as a result of crossings. In these the fundamental chromosome number is eight, and, as in *Rosa*, there is a strong affinity between the homologous chromosomes. Thus the chromosomes of the forms investigated always appear as bivalents in diakinesis and go on the spindle as such.

Blackburn and Harrison (5) examined *Salix* to discover if possible whether variability was of the same sort as in *Rosa*. Diploid, tetraploid, and hexaploid species were found, but in only three cases were abnormalities in meiosis manifested; these were in *S. fragilis*, *S. aurita*, and *S. andersoniana*, and even here the abnormalities were not great. Although they were not so glaring as those characterizing the Rosae, the irregularities were such as to suggest hybridity. Three recognized *Salix* hybrids were examined: *S. viminalis* × *S. purpurea* = *S. rubra* Sm. and *S. caprea* × *S. lanata* gave no disturbance of any sort, not even abortion of pollen grains; while the third, *S. aurita* × *S. phylicifolia*, showed irregular heterotypic division with micronuclei and an irregular homotypic division. In spite of these conditions, the tetrads of the latter cross are not defective and never exceed four in number. Only later is there degeneration resulting in sterile pollen. These investigators thus note that irregularities do not need to be great to suggest hybridity, and the hybrids, *S. viminalis* × *S. purpurea* and *S. caprea* × *S. lanata*, show also that crossing without resulting chromosomal disturbance may occur; while, as shown in *S. aurita* × *S. phylicifolia*, anomalies in division do not preclude the formation of the normal number of microspores, even though sterile pollen may eventually develop.

Cannon (7) very early studied hybrids between different races of peas. Apparently the parents in this case were not very dissimilar, for these race hybrids were fertile, matured their spores regularly, and no abnormal mitoses were observed.

Longley (27, 28, 29) has reported variability associated with polyploidy for *Rubus* and *Crataegus*. This author in a recent paper on *Citrus* (30) finds variability to be general among cultivated diploid forms, and in reference to the variability despite lack of polyploidy in *Citrus*, *Iris*, *Zea* and their relatives, he says: "It seems probable that this variability is the outcome of long cultivation where selection and hybridization would naturally occur." It occurs

to the writer that species of *Wisteria* have been subjected to the same features of selection and hybridization.

The well known work of ROSENBERG (34) on *Drosera obovata* has shown this form to be a hybrid between two parents of different chromosome number, and the resulting chromosomal action is quite unlike anything observed in *Wisteria*.

JÖRGENSEN (24), discussing the chromosome composition and behavior of Danish species of *Callitriche*, decided that hybrids between types with similar chromosome numbers usually do not form dwarf nuclei. This is obviously the condition which obtains in *Wisteria*, and if further investigation on *W. venusta* discloses the chromosomal constitution to be eight, it will form an interesting exception to *Callitriche* conditions, since *W. venusta* divisions frequently allow the formation of more than 4 microspores.

Two *Crepis* crosses involving *C. biennis* (with 20 haploid chromosomes[2]), *C. setosa* (with 4 haploid chromosomes), and *C. capillaris* (with 3 haploid chromosomes) were studied by COLLINS and MANN (10). The cross with the greatest discrepancy in chromosome numbers gave fewer irregularities than the one where a difference of only one chromosome existed, and these authors reported the following conclusion:

> Since the F_1 of *C. setosa* (N=4)×*C. capillaris* (N=3) shows very abnormal reduction phenomena while *C. setosa*×*C. biennis* reduces almost normally, it is evident that normality of reduction does not depend upon similarity of chromosome number, but rather upon likeness of internal composition of the chromosomes.

WINGE (41), in a review of chromosome numbers, reports 8 and 12 as occurring most frequently. To the long list of plants already reported as having 8 chromosomes must now be added various species of *Wisteria*. Concerning hybrid organisms, WINGE says:

> Among plants, specific hybrids are as a rule sterile; indeed, imperfect pollen formation in flowering plants may often be a criterion of their hybrid origin (JUEL 1901 and others) . . . . On the whole, imperfect reduction is associated with the hybrid nature.

If WINGE'S supposition is correct, we might very readily believe from the sterile pollen in *Wisteria* that here we have hybridized

[2] ROSENBERG (36) gives the somatic count for *C. biennis* as 42.

species which have arisen presumably as a result of the propinquity of like-chromosomed parents.

POLYCARY AND POLYSPORY.—*W. venusta* represents the only *Wisteria* species in which polycary and polyspory were observed. There are on record numerous cases where one or both of these phenomena appear in the development of microspores, and in zoological material cases of polyspermy have also been discovered. BEER (2, 3) found supernumerary pollen grains in *Fuchsia* and these resulted from irregular divisions; also he noted a definite relation between the number of chromosomes entering a nucleus, the size of the nucleus, and the size of the cell produced.

Among the Rosaceae, BLACKBURN and HARRISON (4) in their study of *Rosa* counted as many as 8 pollen grains which originated from one mother cell. TÄCKHOLM (37) illustrated a similar condition for *R. canina allodonta*. PENLAND (32) figured polycary in *R. alberti*, and as many as 7 microspores, one binucleate, formed in *R. rubrifolia;* while ROSENBERG (33) gives 10 microspores as resulting from one pollen mother cell. LONGLEY (28, 29) showed polyspory in *Rubus* and in *Crataegus*.

Illustrations of these conditions may be had in *Crepis* hybrids (COLLINS and MANN 10), in *Hemerocallis* (FULLMER 13), in *Nicotiana* (GOODSPEED 14), in *Callitriche* (WINGE 41), etc. HOLMGREN (17) shows that as many as 10 nuclei may be formed from one pollen mother cell in the anthers of peripheral flowers of *Eupatorium glandulosum*.

As noted by JÖRGENSEN (24) for *Callitriche*, the small microspores are usually the first to degenerate, and it is rare that they are able to effect fertilization; indeed they usually perish by the time the shedding stage is reached, and make their contribution to the sterility of pollen. Thus polyspory explains the higher percentage of sterile pollen in *W. venusta* than in the other Asiatic species of *Wisteria*.

The work of the investigators mentioned, and also that of many others (TISCHLER 39; JEFFREY, LONGLEY, and PENLAND 22; LONGLEY 30) makes it apparent that polyspory is the result of irregular chromatin distribution in one or both divisions, and this has come to be regarded as an accompaniment of hybridism.

POLLEN STERILITY.—WULFF (43) considered sterile pollen in plants to be (1) the result of hybridization; (2) an accompaniment of mutation; (3) the results of other influences (temperature, light intensity, moisture, and soil). Of these, hybridization was the most common cause. Since mutating forms have been shown to exhibit cytological phenomena similar to those characterizing hybrids (JEFFREY and HICKS **20, 21**; JEFFREY and ROSCOE **23**), the first two causes given by WULFF may be considered as one.

TISCHLER (38) concluded that "die Pollensterilität kein Specificum des Bastards ist," but one or both parents used in the crossings of *Mirabilis*, *Potentilla*, and *Syringa* which he studied contained sterile pollen, and in the case of *Potentilla* this was accompanied in one parent (*P. tabernaemontani*) by abnormal meiosis; hence comparisons between them and their offspring in regard to pollen sterility cannot well be made. Nor is it necessary to decide that, because the percentage of sterile pollen is increased by subjecting the plants (parents and hybrids) to adverse physiological conditions, pollen sterility, when it occurs in these forms growing under normal circumstances, is not a significant feature.

Reference has been made repeatedly by investigators working with hybrid material, both plant and animal, to the sterility of pollen and of sperms produced. The causal relationship between meiosis and sterility has further been described (WODSEDALEK **42**; ROSENBERG **35**; TÄCKHOLM **37**; HOAR **16**; LONGLEY **28, 29**; BLACKBURN and HARRISON **4**; BEER **2**).

HARRISON'S (15) opinion that sterility is the result of latent hybridity is in accord with the ideas expressed by JEFFREY (18, 19) and his students (FORSAITH **12** and COLE **9**).

From the evidence which we now possess, it seems logical to conclude that when plants growing under normal environmental conditions produce a high percentage of sterile pollen, the explanation may be looked for in previous crossing.

If *Wisteria* species are diploid hybrids, as has been suggested as a possible hypothesis, then despite the apparent compatibility of the chromosomes during meiosis, it seems that sterility may be due to the qualitative differences of the parental chromosomes.

Conclusions

The evidence thus far obtained suggests that *W. venusta*, inasmuch as polyspory is common in the species, may be a somewhat anomalous *Wisteria*. If the supernumerary microspores have been formed here as a result of irregular chromosome distribution, it is probable that they contain fewer than 8 chromosomes, but a study of further material is necessary before any conclusions can be reached. The larger flowers of this form and its vegetative hardiness with the earlier flowering, as well as the additional factors of polycary and polyspory (since these are characteristics common among plants which have resulted from crossings), excite suspicion that *W. venusta* has been derived by hybridization.

Apart from the species just discussed, it has seemed reasonable to compare *Wisteria* with diploid rose hybrids, and with *Salix* hybrids in which crossing has not caused any chromosome disturbance. In the latter genus such hybrids may be entirely fertile. The cross *Salix aurita*×*S. phylicifolia* is even more nearly analogous, for here we have normal microspore formation but eventually sterile pollen.

The genus has been shown to disclose no inconsistency in chromosome numbers. On the contrary, the number seems to be invariably 8. Diakinesis, whenever examined, reveals always 8 pairs constituting firm bivalents, without any suggestion of a loose union of the elements. Any tardiness in reaching the plate, or in the subsequent separation of the chromosomes, is insufficient to cause inequality of the distribution of these units. The action of the chromosomes, however, as for example in *W. floribunda* var. *alba*, displays the sort of tardiness seen in meiotic divisions of hybrids. It is believed that the lack of promptness manifested here is due to the dissimilarity of the elements constituting the bivalents.

Coupled with the chromosome behavior is the still further factor of sterile pollen. Such conclusions as those of MEEHAN (31), who thought there was not "any more sterility attached to hybrids than to ordinary plants," have long since been abandoned.

From the behavior of the chromosomes during the meiosis of the pollen mother cells, and from the presence of varying degrees of sterile pollen, the conclusion is reached that the genus *Wisteria* contains hybridized species.

Summary

1. *Wisteria* pollen mother cells show a perinuclear zone which is more conspicuous after Carnoy fixation than after chromo-acetic.

2. Carnoy's fluid proved preferable for a study of the chromatic figure.

3. Toward the close of prophase the nucleolus becomes vacuolated and loses its intensely basophylic nature.

4. At interkinesis usually more than one nucleolus appears, and three and four are frequently formed.

5. The fundamental chromosome number in *Wisteria* is eight.

6. All the species and varieties examined, both Asiatic and American, show this haploid number.

7. Meiosis proceeds for the most part regularly, although frequently a lack of prompt chromosome action is apparent; this is particularly noticeable in *W. floribunda* var. *alba*.

8. Normal tetraspore formation occurs except in *W. venusta*, where conditions of polycary and polyspory are frequently developed.

9. Asiatic species show varying percentages of pollen sterility. Of these, *W. venusta* develops the highest number of non-functional grains.

10. American species show large amounts of pollen sterility. Comparatively few of the grains reveal cytoplasmic content.

11. *W. venusta* is considered on the basis of polycary, polyspory, and pollen sterility to be of hybrid origin. Other *Wisteria* species with varying degrees of sterile pollen are considered analogous to diploid hybrids in *Rosa* and *Salix*.

12. Pollen sterility is explained as arising from the qualitative differences of the parental chromosomes.

These investigations have been carried on under the direction of Professor E. C. Jeffrey, and I wish here to express my sincere appreciation of his unfailing interest and assistance.

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EXPLANATION OF PLATE VI

W. sinensis Sweet: pollen mother cells during meiosis; magnification approximately 1500.

FIG. 7.—Diakinesis, showing 8 bivalents.

FIG. 8.—Beginning of heterotypic anaphase.

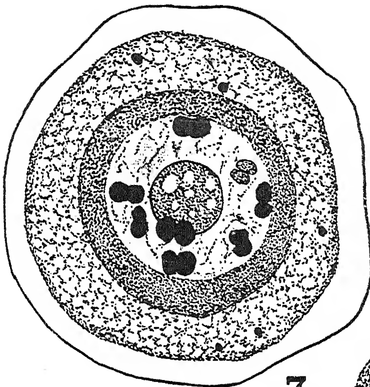
FIG. 9.—Polar view of heterotypic metaphase.

FIG. 10.—Heterotypic metaphase.

FIG. 11.—Interkinesis, showing abundant nucleoli and cytoplasmic masses.

FIG. 12.—Homotypic telophase.

FIG. 13.—Tetraspores.



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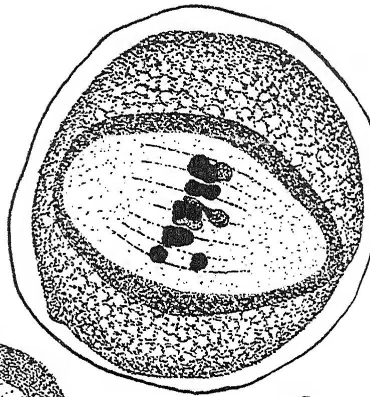

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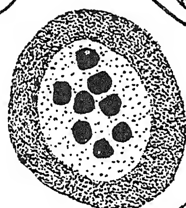

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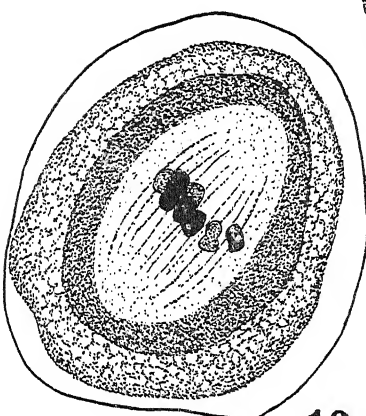

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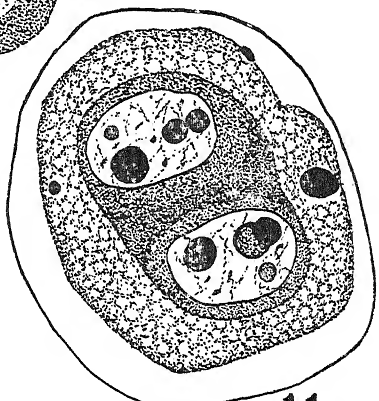

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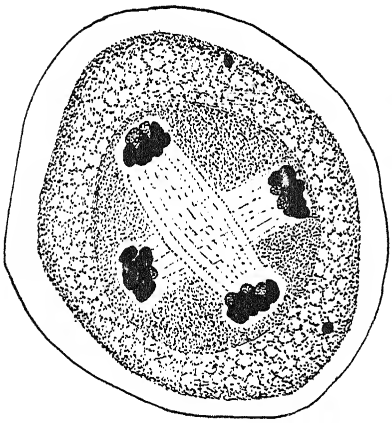

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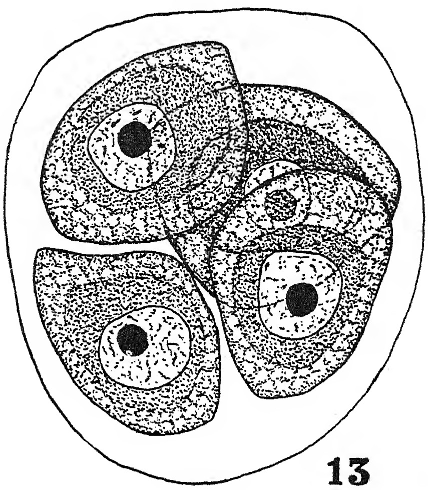

13

ROSCOE on WISTERIA

ANATOMY OF SEEDLING BUDS OF QUERCUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **373**

LaDema Mary Langdon

Introduction

(WITH PLATES VII–IX)

To the present time investigators of the vascular anatomy of the genus *Quercus*, or allied genera, have confined their attention in their search for evidences of conservatism chiefly to the radical, transition region and cotyledonary node of the seedling, or to details of vascular organization in the first annual ring, leaf, node, and root of the adult plant. In these accounts very few and brief are the references to the manner of origin and development of the vascular tissues of the vegetative organs. Believing that an explanation for certain problems relating to nodal anatomy in this genus is more likely to be found in a study of the seedling than in further investigation of mature portions of the plant, the present study of the anatomy of seedling buds has been undertaken. An attempt has been made to determine, for at least two species of *Quercus*, the exact manner of origin, and the course and relation of the vascular elements of the bud scales, leaves, and stipules, so well demonstrated in seedling buds with their close nodal formation; also, in so far as possible, to ascertain the origin of the primary tissues of the stem and the general features of shoot formation.

Material and methods

As a basis for the major part of the investigation, seedlings of *Quercus alba* and *Q. rubra* one to four weeks old, with epicotyls ranging from 1.5–5 cm. in length, have been selected, and serial sections, both transverse and longitudinal, secured through tip portions of the seedlings in such a manner as to include both terminal and axillary buds. For a better understanding of tissue development in the stem, earlier stages in germination (either shortly before or directly after

the emergence of the plumule from the testa and pericarp) were obtained.

The seedlings which furnish the basis for the greater part of this study were from seeds procured in the outlying districts of Chicago, Illinois, the seeds being germinated and the seedlings grown in the botanical greenhouse of the University of Chicago. The preparation and study of the preserved material has been carried out chiefly at Goucher College, Baltimore, and at the botanical laboratory of the Johns Hopkins University.

In dealing either with seedling or winter buds of *Quercus*, great difficulty is likely to be experienced in the preparation of the material, owing to the close overlapping bud scales and numerous glandular hairs on the young stem and foliage leaves. Serial celloidin sections were tested but found to be inferior to paraffin sections where fineness of detail was desired. The seedlings were imbedded in paraffin in the usual manner, care being taken to exhaust all air from tissues before imbedding, thus insuring more perfect infiltration. Sections were then made, either with a rotary microtome or, where dealing with especially refractory tissues, a Spencer sliding microtome. By the latter method a complete series was obtained, by removing each section as cut from the knife, and arranging sections in series upon a slide well coated with albumen fixative and flooded with water. This method has been described in detail in a previous paper (10). Sections were cut 8–10 μ thick. Safranin and light green and Flemming's triple stain were used, the latter combination proving more satisfactory in the differentiating of meristematic tissues.

A typical terminal bud of a seedling, either of *Quercus alba* or *Q. rubra*, consists of several bud scales, numerous stipules, young foliage leaves, and leaf primordia. The stipules may occur as leaf-like appendages arising from either side of the bases of the leaf petioles, or they may occur in pairs without the associated lamina. The latter condition, as pointed out by GOEBEL (4), is occasioned apparently by the arrested development of the lamina of certain of the outermost leaves whose stipules serve as protective organs. Three interesting stages are represented in these seedling buds in the transition from bud scales, which correspond in their development merely to the leaf bases of the more highly segmented foliage leaves, to

foliage leaves where the primordium preserves its normal size and shape. The arrangement and mode of connection with the central cylinder of the vascular elements of the outer paired stipules and bud scales have given rise to two types of nodal structure seldom, if ever, associated with *Quercus*, "unilacunar" and "bilacunar" nodes.

Stem tip

The origin of tissues at the growing point of leaf stems of angiosperms has been the object of numerous investigations, the conclusions from which in the main are contradictory. Literature on this subject dating to 1890 has been so carefully reviewed by DOULIOT (2), that only a brief summary is necessary here, chiefly of works relating directly to the group under discussion. To 1890, according to DOULIOT, one might place in one of two groups authors who had contributed to or concerned themselves with terminal growth in the stems of angiosperms, first those who observed in these plants a single initial cell, and second those who distinguished two or three distinct "histogens."

HOFMEISTER (8), the first to describe an initial cell at the summit of an angiosperm, reported a unique initial in *Zostera marina*, this cell being visible at the beginning of development where it divides as the terminal cell of *Equisetum*. He cited later (9) the maple and ash as having a cuneiform terminal cell, most of the other trees as having a tetrahedronal cell. In 1869 PRINGSHEIM (11) reported a single terminal cell at the stem tip of *Utricularia*, and in 1877 NAEGELI maintained that the leaf of *Elodea* developed, like that of the Cryptogams, by a single terminal initial.

The theory of independent histogens of the stem was introduced by SANIO (1864) and HANSTEIN (1868), and vigorously supported by these authors and by HABERLANDT (5). The researches of HANSTEIN (6) cover a wide field, in all forty-six genera including among the lower dicotyledons *Alnus*, *Populus*, *Platanus*, *Aesculus*, *Sambucus*, *Rhus*, and *Robinia*. He distinguished and illustrated in stem tips of these forms and others three histogenic layers giving rise to the epidermis, cortex, and vascular cylinder. He maintained that these distinct histogens appear at first divisions of the embryo, maintain their independence throughout the life of the plant, and

are increased each by its own group or series of initials. Other investigations on terminal growth in dicotyledons followed these of SANIO and HANSTEIN, some investigators questioning the constancy of occurrence and morphological value of these three layers, a few favoring the idea of a single terminal initial.

The conclusions of DOULIOT appeared, in the main, to support those of HANSTEIN, with less emphasis, however, on the idea of series of initials. He believed that in the great majority of dicotyledons the stem was terminated by three initial cells superposed for the epidermis, the cortex, and the central cylinder, and a small number of others by two initials only; the cortex and central cylinder in this case owing their origin to a single initial. The apetalous dicotyledons, according to DOULIOT, offered two conditions for consideration: (1) the case of two distinct initials, represented by the Urticaceae (*Humulus*), the Polygonaceae (*Polygonum*), the Cupuliferae, (*Carpinus*), and the Begoniaceae (*Begonia*); (2) the case of three initials represented by the Salicaceae (*Salix*, *Populus*).

Anatomical investigations since 1900, whether carried out from the point of view of phylogeny or from that of physiology, have offered slight contribution to our knowledge of the primary origin of tissues in the primitive angiosperms.

Histology of axis

Since differentiation of the primary stem tissues both in *Quercus alba* and *Q. rubra* appears to be essentially the same, *Q. alba* has been selected for detailed description, and reference made to *Q. rubra* only at points of striking similarity or where marked structural differences exist between the two species.

The tip or promeristematic region of the seedling stem of *Q. alba* (epicotyl 2–5 cm.) occupies a zone approximately 0.05–0.06 mm. in length from the extreme tip to the point where it merges into the older meristematic tissues of the stem. This primordial portion is easily distinguished from the older meristematic tissues beneath by its small, uniform, isodiametric and densely protoplasmic cells. At the very apex of this growing point is seen a cell group, consisting of three to four cells which have the character and arrangement of initial or generative cells. In well differentiated material these

initial cells stand out distinctly at the summits of stems, both of *Q. alba* and *Q. rubra*, as four- or five-sided cells (lateral faces four to five in number), from which by tangential (anticlinal) divisions the dermatogen appears to have its origin. Figs. 14 and 18, transverse sections through the stem apex of *Q. alba* and *Q. rubra*, show an arrangement of the superficial rows of cells which points clearly to this apex group as the generative cells, at least of the epidermal layer.

Immediately beneath the initials of the epidermal layer may be seen another initial group, the cells of which (4 or 5 in number) occur in a more or less compactly cylindric or columnar formation. It is this subterminal initial group which in the writer's opinion gives rise to the periblem, in fact to the entire primary meristem, excepting the dermatogen. Periblem and plerome regions are distinguishable in this meristematic section of the stem, but their exact relation to their initial group can be clearly established only by a careful study of the embryonic plumule.

Distinct stem bundles obscure or indistinctly seen in transverse sections of stem tips of older seedlings may be distinguished without difficulty in longitudinal sections of the epicotyl at an early period of its development, especially in stages preceding leaf formation. As evident in fig. 16, a longitudinal section of the vegetative point of the stem axis of *Q. rubra*, the desmogen of the stem or cauline strands is differentiated early, and at a short distance from the lower initial group, apparently by repeated longitudinal divisions in the marginal cells of the plerome.

The epicotyl may reach a length of 1.10 mm. in *Q. rubra* (in *Q. alba*, 0.62–0.70) before the appearance of the first scalelike foliar appendages. After the development of the first four or five bud scales, so close is the succession of leaf primordia at the apex of the stem, and so reduced the extent and prominence of the apical meristematic area of the stem axis, that it becomes increasingly difficult to distinguish cauline strands from the foliar strands or to trace their origin. It becomes a serious question as to whether there are distinct stem or cauline bundles in this genus, for the procambium strands connecting the foliar appendages with the desmogen of the primary axis become the dominant strands of that axis.

A careful examination of serial transverse sections through stem

tips of the older seedlings (epicotyls 2.5–5 cm. in length) discloses no clearly defined desmogen to a point 0.06 mm. below the extreme apex. In this region, where the bases of the youngest leaf primordia merge into the stem tissues, there appear clearly outlined four small, isolated masses of procambial tissue. One of these marks the position of the central procambial or desmogen strand of the youngest leaf primordium. The other three are associated with the primordium of the second youngest leaf. So closely do the leaf primordia succeed one another at the apex of the stem, that their procambial strands appear almost simultaneously in the promeristem of the stem axis.

At the third node from the stem apex, or at a point 10 μ below that described in the preceding paragraph, there may be distinguished in the older meristem five distinct procambial masses, the largest of these being the downward extension of the central petiolar strand of the sixth leaf in order of development; the two stipular strands of this leaf describing partial arcs through the leaf base and cortex to the point where they unite with strands 1 and 3 in the stem.

A close study of nodal topography in the *Q. alba* discloses the fact that these five strands mark the position of the five outward projecting regions of the primary vascular cylinder, and hence indicate the region of entrance into the central cylinder of all leaf traces, both lateral and medium, of subsequently developed leaves. These strands may be followed through the stem a distance of two or three nodes before they merge with other bundles. Gradually the lateral leaf strands swing inward to take their position on the inward projecting points of the cylinder.

At lower levels in the stem the primary cylinder will be found to consist of a gradually increasing number of strands, the more prominent of which are directly traceable in origin to foliage leaves and bud scales enveloping the axis. These foliar strands, to be described in greater detail under leaf structure, have their origin at the bases of the leaf primordia, and in the course of their basipetal differentiation enter the region of the vascular axis, becoming the so-called "common bundles" of the primary cylinder.

No differentiation or development into mature vascular cells can be distinguished in the procambium of the stem tip of *Quercus* to a point 0.09 mm. below the apex of the axis. At this point ligni-

fication of elements is first seen at the innermost points of the older strands of that region. The order of maturation of the xylem elements of these strands is centrifugal.

Leaf

The leaf of *Quercus alba* appears first as a small, conical emergence, at the apex of which is seen a group of two to three cells, the center one of which has the character of an initial cell. At a later stage (fig. 9) there appears in the epidermal layer of this primordium, at either side and several cells removed from the apical group, two slightly elevated cells which mark the point of origin of the two lateral lobes of the primordium. The leaf primordium thus becomes a three-lobed structure which extends around on either side of the stem apex, almost to the base of the preceding leaf. The central lobe of this primordium gives rise, through further development, to the petiole and blade of the leaf, the lateral lobes to the stipules (figs. 7 and 12, also tip of fig. 15).

For a time, or until the young leaf attains a size of from 0.22–0.25 mm., the stipular lobes grow rapidly, not only keeping pace with but exceeding the young blade, arching above that organ as a protection until the leaf apex passes from the embryonic into a permanent condition.

The differentiation of the desmogen or procambial strands takes place at an early period, and is first evident at the base of the leaf primordium. The elements of these strands may be distinguished from the surrounding tissues by the size and shape of the cells, indications of cellular activity and reaction to stains. This differentiation of procambial tissue occurs at three points in the leaf base. The first strand appears in a central position just beneath the central primordial lobe. Two lateral strands arise later, one beneath each of the stipular lobes of the primordium. The progression in development of these three strands is from this point both acropetal and basipetal. Acropetally they enter the three primordial lobes of the leaf, and their further development in this direction keeps pace with the growth of the blade and stipules, the central strand of the three being the middle nerve of the petiole and blade. Basipetally the differentiation of the desmogen strands progresses by repeated longi-

tudinal division of intervening elements of the leaf base and cortex, to the point where they connect with and become a part of the vascular axis. Their relation to and position in the primary cylinder have already been discussed under primary stem tissues.

While differentiation of these first procambial strands is in progress, and before the leaf reaches a size of 0.15 mm., vascular elements for the two lower lobes of the blade appear in the leaf base (fig. 7), one on either side of the central petiolar strand. In their progress through the leaf base toward the stem these lateral procambial strands of the blade meet and unite with desmogen of the stipules (fig. 2), both describing a short arc on either side, through the remaining section of leaf base and cortex, to the point of entrance into the primary cylinder.

With continued growth and expansion of the leaf, and in accord with the acropetal order of evolution of the leaf lobes, other desmogen strands arise from time to time, but in the base of the petiole. Those strands destined for certain of the intermediate lobes of the blade do not enter the vascular axis. In their basal differentiation, there occurs in the upper part of the leaf base an approximation and anastomosis of these lateral strands of the leaf with the stipular vascular elements described in the preceding paragraph. This is clearly illustrated in transverse sections in figs. 2, 3, and 4.

The vascular elements of the upper lobes of the leaf do not unite, either with the central petiolar strand or with the lateral blade and petiolar strands, but maintain a direct and independent course in their basipetal progression, entering the primary cylinder one on either side of the central petiolar strand. Thus the median leaf trace of *Quercus* (in the fully developed leaf) is seen to consist of three bundles, and the nodal structure of the typical leaf is described as trilacunar (leaf traces three to five in number with three gaps in the central cylinder, one median and two lateral).

The first lignification or thickening of the procambial elements appears in the cells of the leaf base, and progresses with the advance of these elements inward toward the stem and outward and upward toward the leaf tip. The first formed central strand of fig. 7 shows two, possibly three cells, apparently of the protoxylem, undergoing

thickening. There are no distinct phloem elements distinguishable until a later period (fig. 19).

Bud scales

As previously noted, the first foliar appendages developed on the stem axis either of *Q. alba* or *Q. rubra* are not foliage but scale leaves (bud scales). These appear when the epicotyl reaches a height of 1.0–1.1 mm. Each young bud scale has four well defined desmogen strands which, like the procambial strands of the foliage leaves, have their origin at the bases of the appendages. These strands merge in pairs, and as two strands they approach the vascular axis.

The characteristic nodal structure at the point of insertion of the outer scalelike leaves or bud scales of *Q. alba* is shown in fig. 6, a transverse section through the basal portion of the plumule of one of the older seedlings. The bud scales illustrated have each at their base four vascular strands, each strand consisting chiefly of protoxylem elements. These strands unite in pairs in the base of the bud scale, forming two bundles, and in this condition they approach and enter the vascular axis both at the same point, thus causing but one gap in the primary cylinder. It is evident, therefore, that the first formed seedling nodes of *Quercus* are not of the trilacunar type usually associated with this genus.

Fig. 5, a transverse section through a portion of the same seedling as illustrated in fig. 6, but at a slightly higher level, shows the manner of connection of the vascular elements of the outer paired stipules with those of the central cylinder. Attention is called to the fact that each of these stipules has three strands which, like those of the leaf, have their origin in the bases of these stipular appendages. Near the point of connection with the stem the two stipules unite in a common base, their vascular strands meeting and uniting in pairs, thus forming three prominent bundles. These three bundles connect with the primary cylinder at two points, two of the three forming the median leaf trace, the third as a single lateral strand describing a partial arc through the leaf base to the lateral leaf gap.

Discussion

Attention is directed in particular to the variable nodal anatomy exhibited in the seedling buds of the two species of *Quercus* examined. From the inner foliage leaves to the outer paired stipules and bud scales, there are evident three distinct types of nodal structure: (1) trilacunar (leaf traces three to five in number with three gaps in the central cylinder, one median and two lateral), characteristic of the foliage leaves; (2) bilacunar (leaf traces three in number with two leaf gaps, one median and one lateral), characteristic nodal condition at the point of connection of the outer paired stipules with the stem; and (3) unilacunar (two-strand vascular supply with but one gap in the vascular cylinder), found at point of insertion of the outer bud scales. In view of the facts here outlined, the question arises whether this simple unilacunar condition characteristic of the first formed leaves of the oak seedlings is to be regarded as a primitive character, or does it simply illustrate a case of reduction from the trilacunar type by the complete disappearance of the two lateral traces and gaps? Observations made in this investigation tend to support the former idea.

Facts brought forward thus far in the study of the origin of the vascular strands of the leaves indicate that the topography of the node, whether unilacunar, trilacunar, or multilacunar, is dependent upon or determined by the character of the organ developed at that node, rather than the reverse. Investigations based on the theory that the character of the node determines the nature of the nodal appendage (13) have failed to take into serious consideration the point of origin of the leaf strands, but appear to be guided by the vague but generally accepted theory that the leaf traces are put forth from the vascular cylinder, and that these developing traces exert a peculiar influence on the growing leaf primordia.

A recent text on plant anatomy (3) defines leaf traces as prolongations of the stelar vascular supply extending into the leaves, and states further:

> The term leaf trace is used in two somewhat different ways, as applied to any bundle which extends to a leaf, and to the complex of bundles which supply a given leaf. Since the trace is merely an extension of the vascular system of the

stem, either as a definite abruptly separated branch of that system, or as a strand gradually set off as a distinct part, there is not usually a definite point of origin of a leaf trace.

In the oak, the differentiation of the cells composing the leaf traces commences in the leaf base, and progresses from this point both outward toward the leaf tip and inward toward the central cylinder. The vascular strands, therefore, do not determine the character of the developing structures of the seedling but the primordia of the developing leaves or leaflike appendages start, and the vascular elements are differentiated in the tissues of these growing organs. Much the same idea has been suggested by Coulter and Land (1) in their study of the dicotyledonous embryo of *Agapanthus umbellatus*.

Summary

1. The primary stem of *Quercus alba* and *Q. rubra* shows two distinct initial groups, one for the dermatogen and a second (subterminal) common to both periblem and plerome. Distinct cauline strands may be distinguished in the primary tissues of the epicotyl only at an early stage of seedling development; such strands apparently taking their origin just beneath the initial regions and from the marginal cells of the plerome.

2. With the appearance of leaves or leaflike appendages at the stem tip, the vascular elements of these members (the foliar strands) become the dominant bundles of the primary cylinder. This fact no doubt has led to the commonly accepted idea that the primary cylinder of the more ancient dicotyledons consists only of bundles of the leaf trace.

3. The tissues of the leaf appear to originate from two initials: one for the epidermis, the second for the parenchyma and the bundles.

4. The procambial strands of the foliar organs originate in the bases of the leaf primordia. Differentiation of these strands progresses from the point of origin both basipetally and acropetally. The first thickening of xylem elements occurs in the procambial tissue in this region.

5. Both species of *Quercus* examined furnish evidence of variable nodal structure, the character of the node being largely determined by the nature of the developing foliar organs.

The writer wishes to express thanks to Professor CHARLES J. CHAMBERLAIN and Dr. W. J. G. LAND for kindly criticism and encouragement throughout the progress of the work; and also to Professor DUNCAN S. JOHNSON for many courtesies received while working in the Johns Hopkins Laboratory.

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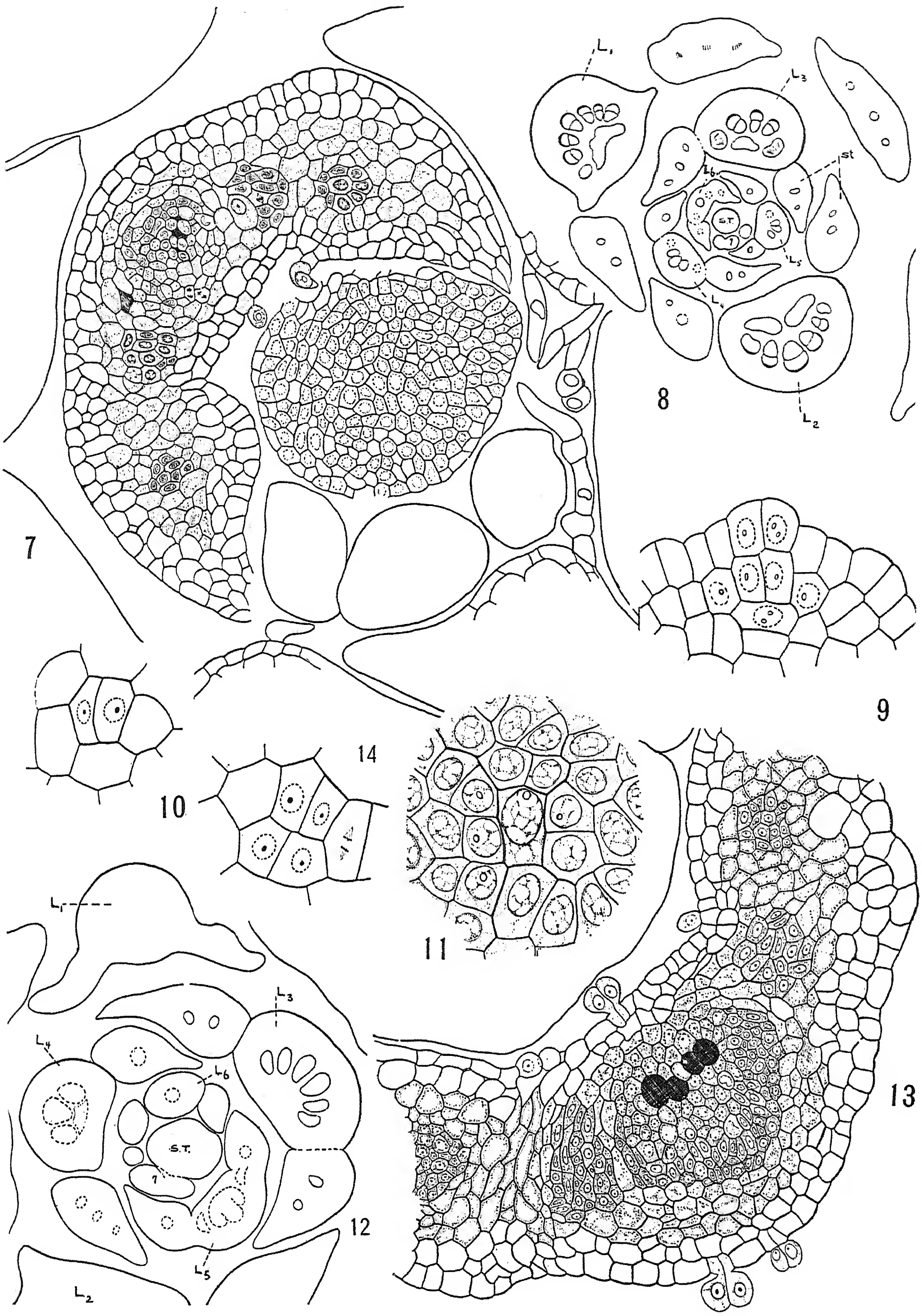
LANGDON on QUERCUS



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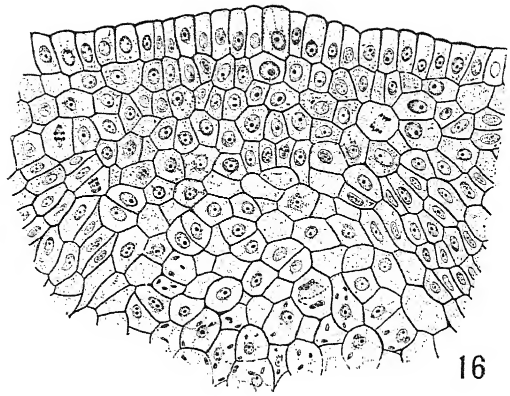

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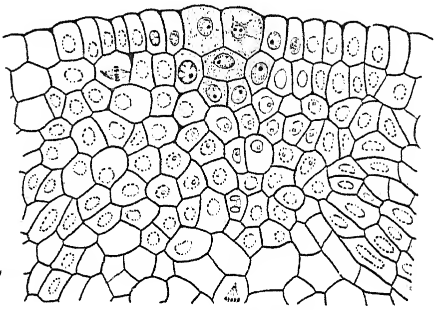

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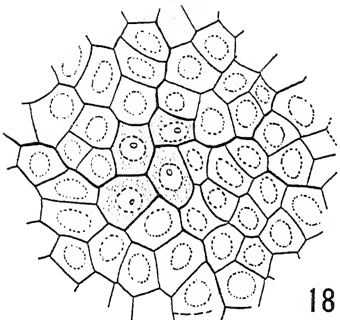

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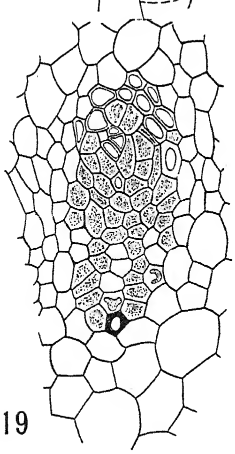

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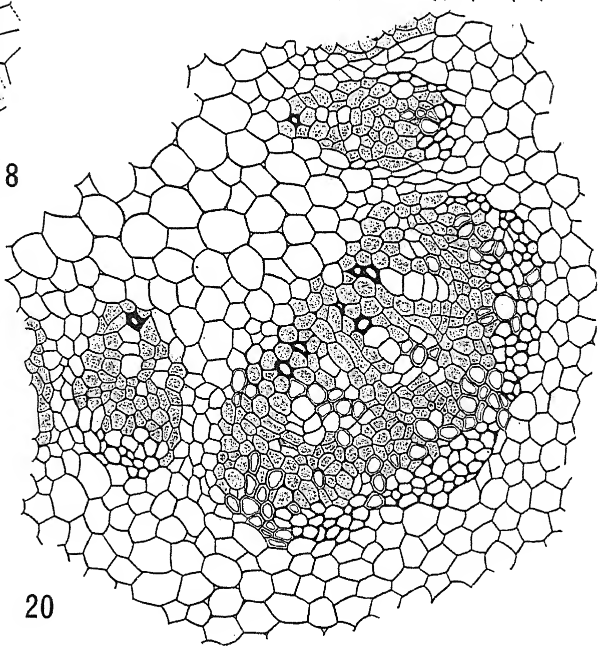

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LANGDON on QUERCUS

EXPLANATION OF PLATES VII–IX

PLATE VII

FIG. 1.—Transverse section through tip of epicotyl, showing arrangement of petioles and stipules of foliage leaves.

FIG. 2.—Section through tip of same seedling as fig. 1, at lower level; connection of stipules with base of petiole shown in younger leaves, also relation of stipular and petiolar vascular supply.

FIG. 3.—Manner of connection of vascular strands of foliage leaves with stem cylinder.

FIG. 4.—Same seedling, at 30 μ below point illustrated in fig. 3.

FIG. 5.—Transverse section illustrating manner of connection of vascular elements of outer paired stipules with those of central cylinder.

FIG. 6.—Characteristic nodal structure at point of insertion of outer scale-like leaves or bud scales; figs. 1–6, $\times 48$.

PLATE VIII

FIG. 7.—Detail of leaf no. 6, illustrated in outline in fig. 8; $\times 352$.

FIG. 8.—Transverse section through tip of epicotyl at point where stem apex first appears: L_1, L_2, L_3, etc., foliage leaves numbered according to order of development; *ST*, stem tip; *st*, stipules; $\times 48$.

FIG. 9.—Section through young leaf primordium; $\times 760$.

FIG. 10.—Tip of stipule of embryonic leaf; $\times 912$.

FIG. 11.—Tip of petiolar lobe of embryonic leaf, showing epidermal initial; $\times 912$.

FIG. 12.—Transverse section of seedling tip; $\times 80$.

FIG. 13.—Detail of fifth leaf, illustrated in outline in fig. 12; five procambial strands shown at base of petiole, three center strands merging, and one procambial strand at base of each stipule; $\times 352$.

FIG. 14.—*Q. rubra:* stem apex showing epidermal initials; $\times 912$.

PLATE IX

FIG. 15.—*Q. alba:* longitudinal section of young epicotyl: *bs*, bud scales; *pr*, primordium of foliage leaf; $\times 48$.

FIG. 16.—*Q. rubra:* longitudinal section of portion of stem apex; $\times 352$.

FIG. 17.—*Q. rubra:* longitudinal section of portion of stem apex; $\times 352$.

FIG. 18.—*Q. alba:* transverse section of stem apex showing epidermal initials; $\times 760$.

FIG. 19.—Cross-section of young bundle; enlarged cells just beyond xylem elements cambium initials; $\times 456$.

FIG. 20.—*Q. alba:* transverse section of stem of seedling near point of connection of scale leaves with stem; shows detail of primary cylinder at level in stem illustrated in fig. 6; $\times 352$.

A CORKY-BARKED MUTATION OF HEVEA BRASILIENSIS[1]

HARLEY HARRIS BARTLETT

(WITH SEVEN FIGURES)

This paper reports a new type of rubber tree in which true cork is produced from a cork cambium. *Hevea brasiliensis* mut. **granthami** (=**Hevea granthami**), mut. nov., differs from all other forms of the highly variable plantation rubber of the Far East in the production from a cork cambium of true cork, which appears in longitudinally arranged patches and ridges on young stems about one year old, and eventually attains a thickness of 16 mm. on the trunk (figs. 1, 2). Type, the original seedling, on Tanah Radja Estate of the United States Rubber Company, Asahan, East Coast of Sumatra; vegetatively propagated in Sumatra and Java. (Museum specimens, *Bartlett* 8747, H.A.P.M., Asahan, Sumatra, July, 1927, in Herb. Univ. Mich. & U.S. Nat. Herb.)

The largest area of plantation rubber under single management in the world is that of the Hollandsch-Amerikaansche Plantage Maatschapij (usually referred to in the East as H.A.P.M.) in Asahan, East Coast of Sumatra. H.A.P.M. is a subsidiary of the United States Rubber Company. Here had been planted, up to the year 1920, 5,000,000 trees. Since then over a million more have been set out. All of the trees have been continuously under scientifically supervised observation, since they have been utilized in a vast selection experiment, the object of which, of course, has been the acquisition of high yielding clones for budding, and of superior stocks upon which to bud them. In this selection work, without doubt the most extensive operation ever carried out for the improvement of a tree crop, a great many interesting variations of *Hevea* have come to light.

To the general botanist, who is not concerned with the economic problems involved, the most interesting tree among the 6,000,000 is

[1] Papers from the Department of Botany of the University of Michigan, no. 257.

the one which it is proposed to call *Hevea brasiliensis* mut. *granthami*, in honor of JAMES GRANTHAM, Director of the Plantation Research Department of the United States Rubber Company. In the

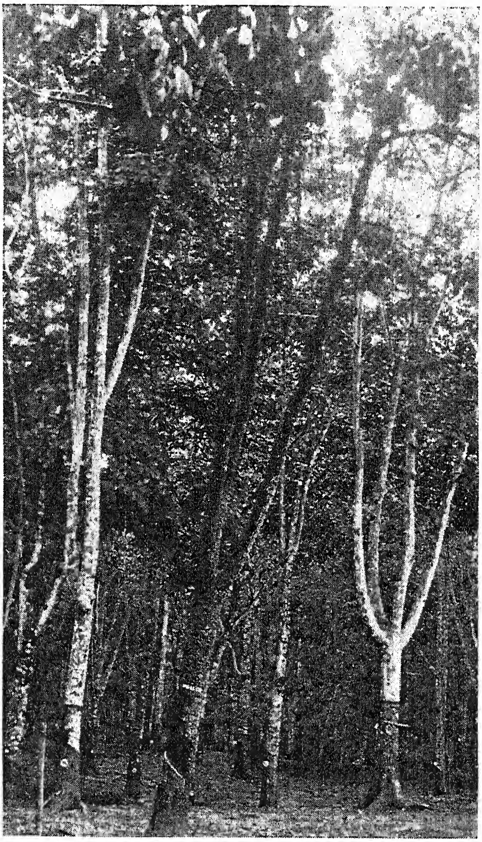

FIG. 1.—Type tree of *Hevea brasiliensis* mut. *granthami*, surrounded by ordinary smooth-barked trees; Tanah Radja Estate, Asahan, East Coast of Sumatra (Phot. H.H.B.).

preceding description *Hevea granthami* is suggested as an alternative name. If the origin of the new type were unknown, most botanists would consider it a distinct species. Those who give binomial names freely to every important type of woody plant will doubtless prefer the simpler nomenclature.

On account of the attention which has been directed to the single original tree of mut. *granthami* and its vegetative progeny, it may be stated with considerable certainty that there has been no other individual like it among the 6,000,000 trees on H.A.P.M. Furthermore, since various other estates beside those of H.A.P.M. are under the control of the United States Rubber Company, a large additional number of trees is known to include none of the new type. It is therefore of the highest rarity. The writer interprets it as a seed mutation sufficiently diverse from the parent to rank as a distinct species.

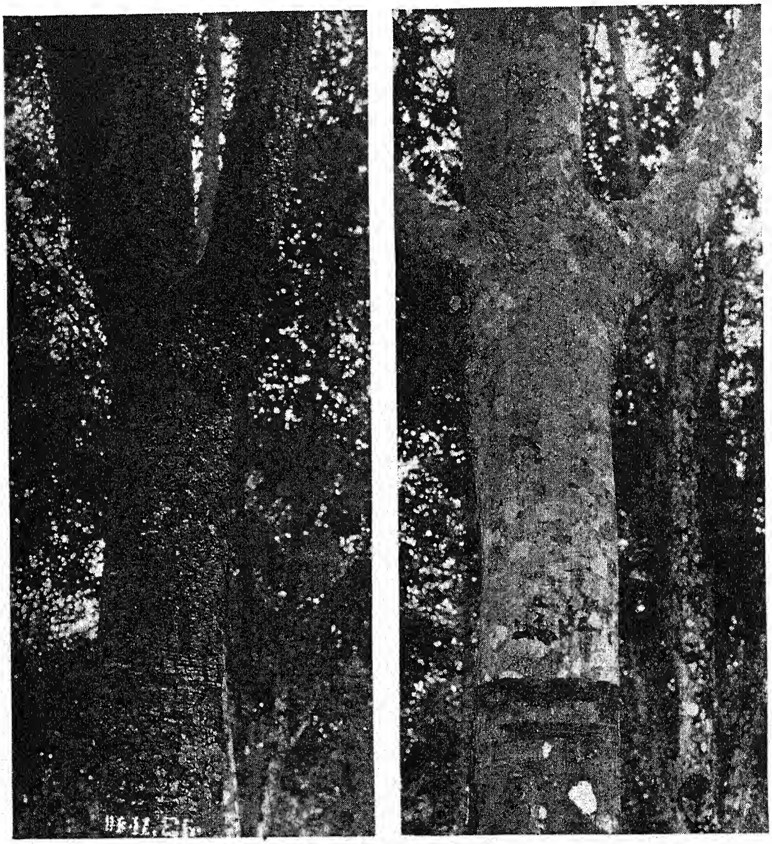

FIG. 2.—Trunk of type tree of mut. *granthami*, above tapping area (left), and corresponding picture of ordinary tree of same age and size (right); in mut. *granthami* the cork extends to the small branches, and is very conspicuous on trunk (Phot. H.H.B.).

Of course the status of the new form as a mutation rather than as a Mendelian segregate or recombination is subject to the usual criticisms, and at the present time is quite incapable of proof. Since parallel types occur in nature in other genera of woody plants, even the probability of a mutational origin is of no little interest from a general systematic and evolutionary standpoint.

Fig. 3

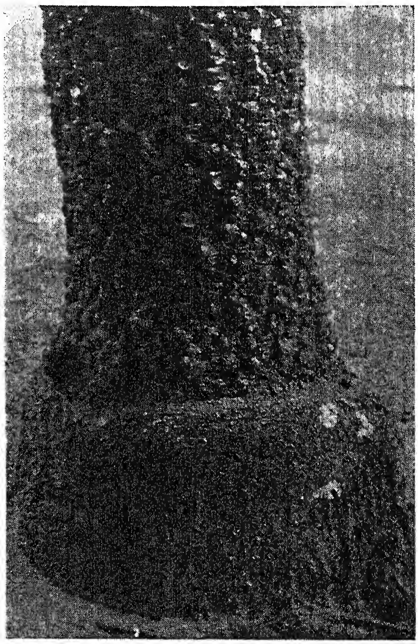

Fig. 4

Figs. 3, 4.—Fig. 3, high budding of mut. *granthami* on ordinary smooth-barked stock (Phot. H.H.B.); fig. 4, low budding of mut. *granthami* on rough-barked but not corky seedling; roughness of stock due to fissuring of coarse thick bark (Phot. H.H.B.).

The best known cork-bearing tree, of course, is the Mediterranean cork oak. It would have been a bold mutationist indeed who would have ventured to hint at the derivation of such an apparently highly specialized type from a non-corky precursor by a single mutation, without being able to cite such a case as that of the sudden

appearance of *Hevea brasiliensis* mut. *granthami*. The writer ventures to predict that the mutational origin of corkiness in *Ulmus* might be observed without growing an excessive number of seedlings, since the character seems to have appeared many times in the evolution

FIG. 5

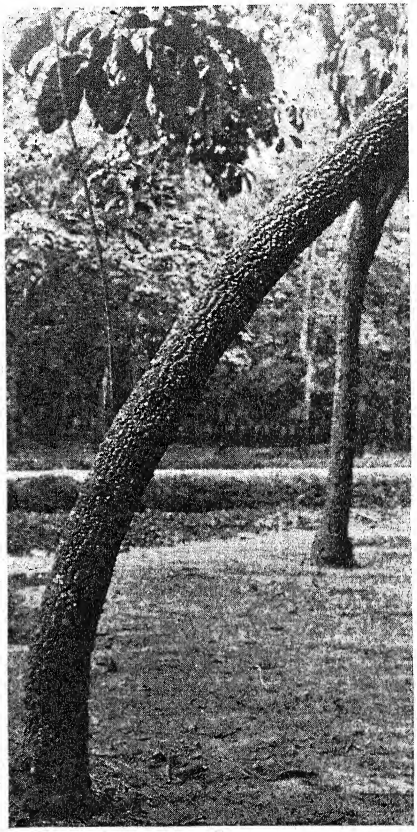

FIG. 6

FIGS. 5, 6.—Fig. 5, rough-barked ordinary seedling of *Hevea brasiliensis* which has been twice budded, once near base with a very smooth-barked type, and again on smooth scion with mut. *granthami;* the photograph demonstrates the hereditary distinctness of the three types; the stock has a dead, fissured outer bark but no cork; smooth-barked scion has only living bark, for there is a layer of green cells under the whitish epidermis; upper scion of mut. *granthami* has a cork cambium and abundant cork development (Phot. H.H.B.); fig. 6, low budding of mut. *granthami* grown without artificial support; tree has drooped over in a manner characteristic for the whole lot of over 130 buddings (Phot. H.H.B.).

of the species of this genus. Whether or not species of *Hevea* other than *H. brasiliensis* develop true cork the writer does not

know, and, writing from Sumatra, is not in a position to look into the matter.

The history of the *Hevea* rubber of the Malayan region is well known, and has recently been again reviewed by MAAS.[2] It is clear that all the trees of H.A.P.M. are descended from WICKHAM's original introduction of 1876. The seedlings of the first generation were sent to Singapore from Kew. In 1883 and subsequently they bore seed, from which a second generation was grown. The pioneer estates of Malaya were initially stocked from the seeds of the second generation. The H.A.P.M. received most of its original stock from Cicely Estate near Teluk Anson, and Vallambrosa Estate between Kĕlang and Kuala Selangor. The later plantings at these estates were from seeds produced by their own trees, so the original H.A.P.M. stock belonged to the third and fourth generations from the WICKHAM introduction.

With regard to roughness of bark, there is a very considerable variation among rubber trees (figs. 3, 4). However, the common type of roughness is due to the fissuring of the dead outer layers of unusually thick bark, and does not extend far up the trunk. A very few seedlings develop cork on the main trunk, but not at all to the degree that characterizes mut. *granthami*, and not on the young growth. In mut. *granthami* the cork develops from a cork cambium just below the epidermis. It appears first in longitudinal patches with cross checkering. These patches become confluent and form irregular ridges by the gradual lateral differentiation of the cork cambium. The cork peels off very readily, disclosing the smooth surface of the cork cambium, which is scarlet on the main trunk of the original tree and varies to yellow on the smaller trunks of the vegetative progeny.

The original tree has had the lower part of the trunk repeatedly tapped. The renewed bark on the tapped area has developed a cork cambium near the surface, on which there is a sheet of cork 1 cm. thick. The cork on renewed bark has a smooth, irregularly checkered surface, since the cambium from which it grows is differentiated at once over the tapped area, and not in originally isolated longitudinal strips, as on virgin bark.

[2] MAAS, J. G. J. A., Het tapsysteem van *Hevea brasiliensis* op proefonderfindelijken grondslag. Arch. Rubbercult. 9:1–221. 1925 (pp. 11–17).

The original tree of mut. *granthami* first came to notice because of its high yield of latex, which led to its being selected as a mother tree from which to make buddings. The numerous budded trees soon showed uniformly the cork ridges above the unions with the ordinary stocks (fig. 5). The genetic individuality of *Hevea* trees is only fully realized when large numbers of budded trees of the same age, strikingly identical, are compared with a block of seedlings. It then becomes obvious how large is the factor of heredity, and how

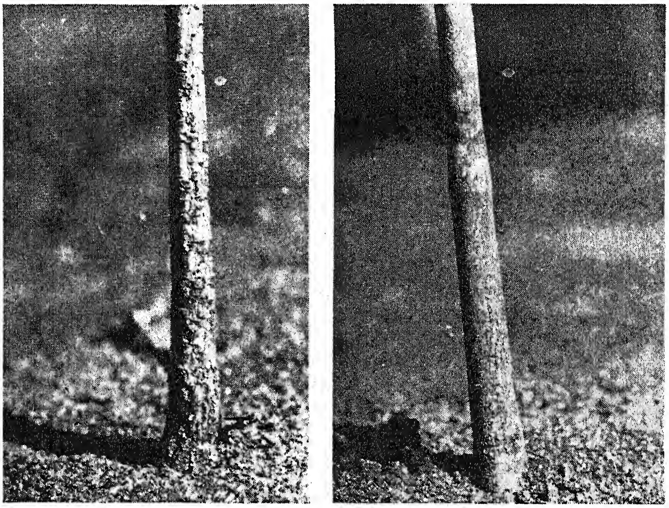

FIG. 7.—Young seedlings of mut. *granthami* (left) and ordinary *Hevea brasiliensis* of same size and age (right) (Phot. J. GRANTHAM).

relatively insignificant is the factor of environment in bringing about the diversity within an ordinary stand of seedlings. The buddings of mut. *granthami* proved its great distinctness from all other types, and led to an unsuccessful search of the plantations for other individuals.

Buddings of mut. *granthami* have the peculiarity that the trunk does not at first grow rapidly enough in diameter to support the heavy foliage. As a result all the trees bend over unless given artificial support (fig. 6). There is no reason to believe that this characteristic is necessarily associated with corky bark.

Approximately 200 seedlings have been grown from unguarded seeds of the original tree of mut. *granthami*. Three of them repeat the characteristic of the parent (fig. 7). Since most seeds of *Hevea* are the result of cross-pollination, it is a fair supposition that mut. *granthami* is a recessive. The three seedlings that have come true perhaps represent self-pollinations, and the remainder cross-pollinations. The three seedlings display thick cork on bark only a few months old.

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A STUDY OF PREISSIA QUADRATA

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(WITH FIFTEEN FIGURES)

The adult gametophyte of *Preissia quadrata* almost rivals in complexity of structure that of its near relative, *Marchantia polymorpha*. Results obtained from a study of spore germination and gametophyte development in *Marchantia*[1] suggested a similar investigation in *Preissia*. HAUPT[2] says that a functioning apical cell does not occur in the embryo of the Marchantiales; however, he describes and figures a single cuneate apical cell for the adult thallus of *P. quadrata*, and also for *Reboulia hemisphaerica*.[3]

Material

The material for this study was collected at Sinsinawa Mound, Grant County, Wisconsin, some in the autumns of 1924 and 1925, and more in the spring of 1926. The first consideration will be with the plants as they were found in this locality about October 10. As stated by HAUPT and some of his predecessors, *Preissia* is not only a monoecious plant, but there also occur mixed heads. A longitudinal section (fig. 1) of one of these plants shows the two-sided character of some of these heads. Of about ten heads that were apparently male, two proved to be mixed when sectioned. The other one of these showed a rather well developed sporophyte. It was at least as well developed as any of the embryonic sporophytes were at the close of the growing season. Sections of a typical male receptacle taken at right angles to the vertical axis of the stalk show that there are about thirty-six to forty antheridia in a single receptacle. A transverse section of a thallus bearing an archegonial head (fig. 2) shows the autumn condition of the young sporophytes. Although a group of three young sporophytes is seen at the left of the figure, there is a

[1] O'HANLON, SISTER MARY ELLEN, BOT. GAZ. 82:215–222. 1926.

[2] HAUPT, A. W., BOT. GAZ. 82:30–54. 1926.

[3] ———, BOT. GAZ. 71:61–74. 1921.

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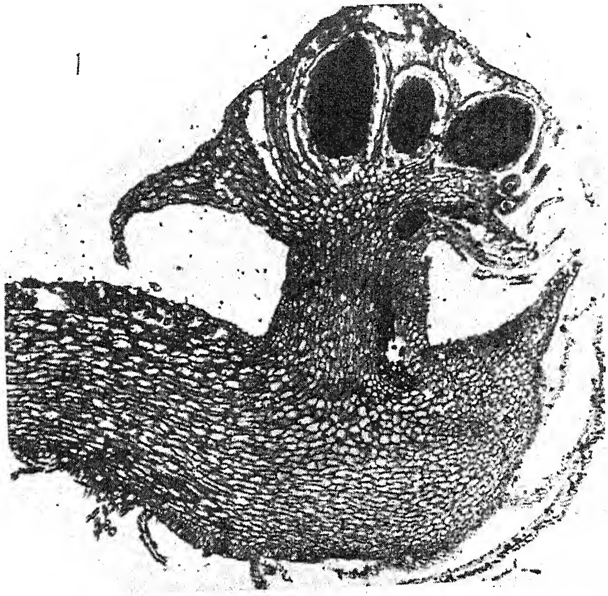

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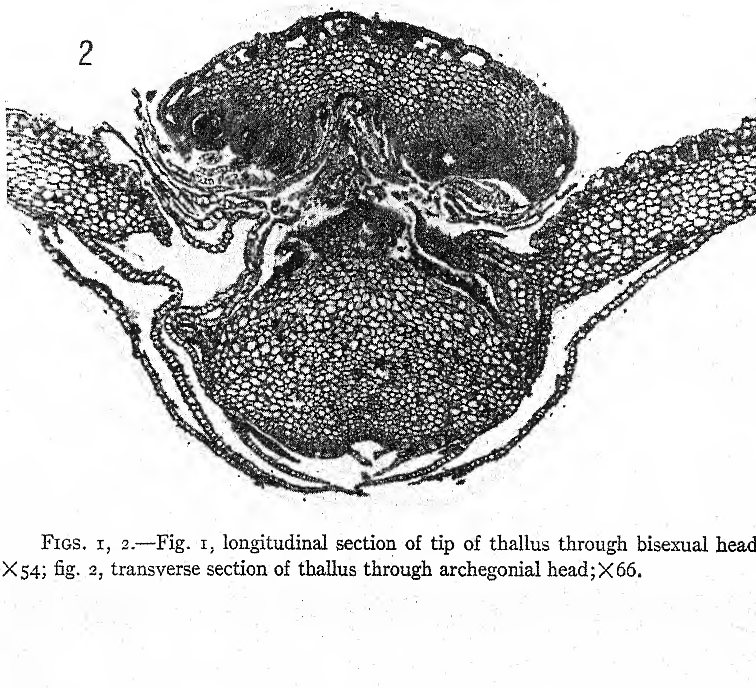


FIGS. 1, 2.—Fig. 1, longitudinal section of tip of thallus through bisexual head; ×54; fig. 2, transverse section of thallus through archegonial head; ×66.

question whether it often occurs that more than two sporophytes in a single quadrant ever come to maturity.

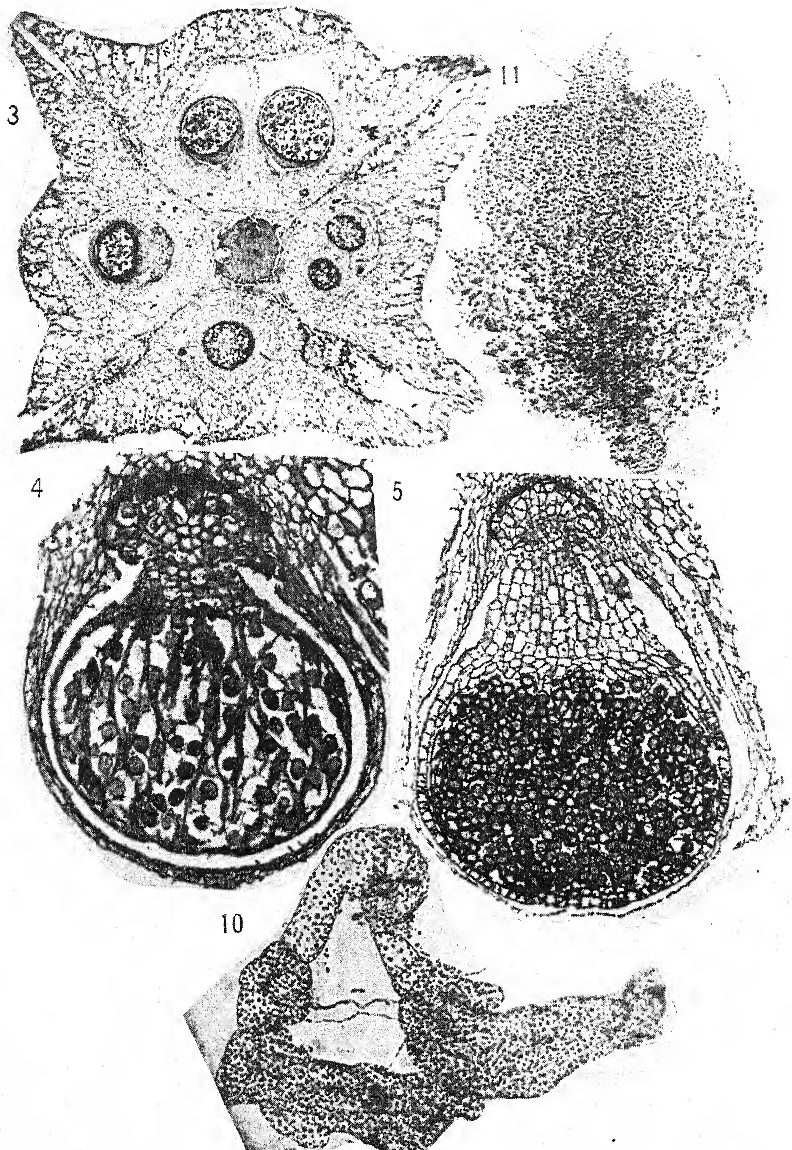
Plants collected May 10, and which reached the laboratory on the twelfth of the month, were more or less uniformly in the condition which is illustrated by a horizontal section through the archegonial receptacle (fig. 3). The number of archegonia in a quadrant ranges from three to six; the dominant number, the largest shown in this figure, is four. The average number of archegonia to a head probably does not exceed sixteen.[4]

The intracapsular tissue of the young sporophyte, found in most of the plants at this season, was differentiated into spore mother cells and elaters in an incipient stage of metamorphosis. Although the development of the sporophyte and the genesis of the spores were not sufficiently investigated to warrant a confident statement, it seems that the spore mother cells and the elaters of *Preissia* are of the same generation. This deduction is based largely on the comparative volume of the incipient elaters and that of the spore mother cells, as shown in fig. 6 *a*, as well as of sections, one of which (fig. 4) is a photomicrograph. At any rate, the ratio between the number of spore mother cells and the number of elaters is much less in *Preissia* than it is in *Marchantia* (figs. 5–6 *a-e*). Fig. 5 is a median longitudinal section of a mature sporophyte. In the laboratory, the period between the spore mother cell stage and that of the mature spore stage was about a week. Although fertilization probably occurs in every archegonium, the greatest number of mature sporophytes that were counted in any head was six, with not more than two to a quadrant.[5] In an examination of over one hundred heads, the range was from one to six, with an average of 2.98, and a dominant number of four sporophytes to a head. The average number of mature sporophytes in a head of *Marchantia polymorpha* was estimated at twenty-four.

The number of spores in a capsule of *Preissia* is estimated at 3000, with an average of nearly 9000 to a head. This is a marked re-

[4] The number of archegonia in a single group in *Marchantia polymorpha* is sometimes sixteen or over, with an average of eight groups to a receptacle.

[5] Many of the foregoing statements concerning *Preissia* are not new to the student of the Hepaticae and are merely repetitions of the facts stated by previous writers, including HAUPT, who has adequately cited and discussed the literature which is pertinent to this subject.



FIGS. 3–5, 10, 11.—Fig. 3, horizontal section through archegonial receptacle; ×24; fig. 4, median longitudinal section of young sporophyte; ×60; fig. 5, median longitudinal section of mature sporophyte; ×65; fig. 10, twin thalli; ×54; fig. 11, young gametophyte showing apical notch, mucilage hairs, and thickened midbody region; ×178.

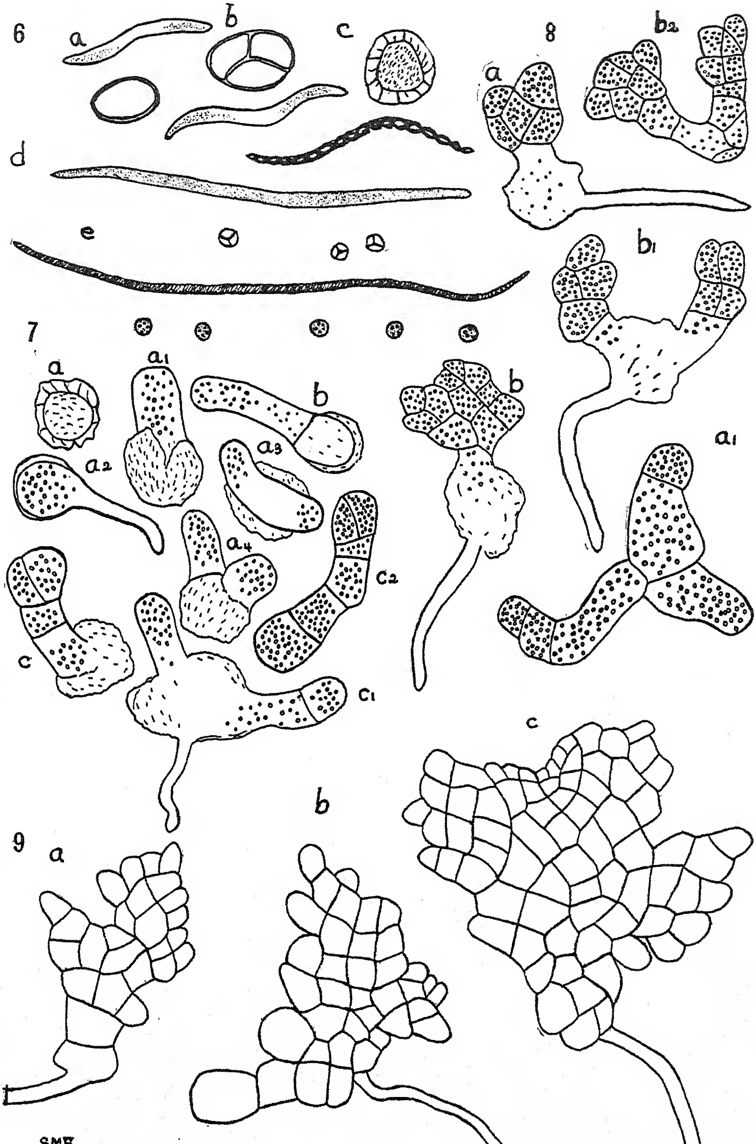
duction when comparison is made with *Marchantia*, in which the spore output for a single head is probably over 7,000,000. Notwithstanding this fact, however, when we consider the volume of the sporogenous tissue in the two genera, what seems to be such a striking advance in the case of *Preissia* is, after all, more apparent than real. It is true that the number of spores is greatly diminished in *Preissia*, and that the elaters are more numerous in proportion to the number of spores than in *Marchantia;* but a comparison of the size of the spores and the elaters of the two genera is disturbing to the theory that there is so much progress in the sporophyte of *Preissia* (fig. 6). The diameter of the ripe spore of *Preissia* is 75 μ. The diameter of the spore of *Marchantia polymorpha* is not over 18 μ. The elaters, although less numerous in proportion to the number of spores in a single capsule of *Marchantia*, still greatly outnumber the elaters in a sporophyte of *Preissia*, to say nothing of their greater volume. It is plain, therefore, that the real gain in the reduction of fertile tissue made by *Preissia* over its well known congener is in the paucity of the number of sporophytes, rather than in the actual reduction of the volume of fundamentally sporogenous tissue within the capsule itself.

Spore germination

On May 19 some of the material which was collected May 10 was still in the spore mother cell stage, some was in the tetrad stage, while most of it was in the mature condition. On this date spores in all three stages of development were sown on porcelain plates kept moist with a mineral nutrient solution in glass chambers. These cultures were set up in an east window of a white walled room with north, south, and east exposures. The response made by the unripe spores was nil, but germination was evident in the ripe spores in about six days from the date they were sown, and it was 100 per cent. Tests were made on spores from the same collection between four and five months later, with results that showed persistent viability in about 10 per cent of the spores.[6]

Fig. 8 shows the variety of initial steps in spore germination. Probably there most frequently occurs the protrusion of a short germ tube as typified in *a;* often a rhizoid makes the first step as in a_2

[6] The spores of *Marchantia polymorpha* are 100 per cent viable for over a year.



FIGS. 6–9.—Fig. 6, spore mother cell and incipient elater, tetrad and more advanced elater, mature spore and elater, of *Preissia;* tetrad and ripe spore with corresponding stages of elater of *Marchantia polymorpha;* ×132; fig. 7, mature spore and early stages in spore germination; ×132; fig. 8, young gametophytes; ×132; fig. 9, later stages in development of young gametophyte; ×132.

and, less frequently perhaps, there appear simultaneously two cells which introduce a branching or twin condition (a_3–a_4). Sometimes there appears in the same culture a kind of filament not greatly unlike an algal form or moss protonema, as c_2. Experiments made with sowings on a liquid medium and in weak light gave even less satisfactory results than those which were obtained under similar conditions with *Marchantia* spores. It would seem that *Preissia* is even less inclined to a hydrophytic habit than *Marchantia* in these early stages, and of course is consistently more strictly mesophytic in its adult behavior.

One of the most interesting features in this study is the difference in the size of the cells in the sporelings of *Preissia* and *Marchantia*. The greater size of the cells in the sporeling of *Preissia* is accounted for by its much greater spore size. In the progress of the development of the young gametophyte, however, the size of the cells is gradually diminished so that in the adult thallus of *Preissia*, and, even long before that stage, the cells are no larger than those of *Marchantia*, perhaps, not even so large.

Young gametophyte

Fig. 8 shows a series of stages in the development of the young gametophyte. The decrease in the size of the cells is due to the rapidity with which the successive mitoses occur. There is relatively little time for growth between each two mitotic divisions, and consequently there is rapid progress in the development of the young gametophyte. More advanced stages are seen in fig. 9. In *Preissia*, as in *Marchantia*, there always appears a distinct apex with its row of meristematic cells in at least one region of every progressive young thallus. When branching occurs, two or more such regions are established even very early (fig. 10). No more in *Preissia* than in *Marchantia* can we say that there is a single apical cell functional in the development of the young plant or plants which grow from a single spore that may be designated as *the* apical cell.

Since in *Preissia* there is no definite midrib in the adult thallus, cell division in the third plane of the young gametophyte is less restricted, making the thickened area more extensive than is the case in *Marchantia* at a corresponding stage of development (fig.

11). Contrary to the conditions in *Marchantia*, there are no special cells for the storage of essential oils in the young gametophyte of *Preissia*. The general contour of the young thallus also is more irregular than that of *Marchantia*. Anchorage is by mucilage hairs and rhizoids. The rhizoids of the young plants are of the plain walled type, as in *Marchantia*. Thus it is seen that the young gametophytes of these two genera are more or less similar in origin and structure, and seem to share certain resemblances with fern prothallia. There are some very obstinate and fundamental differences, however, even as there are in the adult plants.

Adult thallus

A transverse section of the adult thallus (fig. 12), somewhat behind the apex, shows the thickness of the plant. There are thirty-five or more cells, exclusive of the chlorophyllose cells in the air chambers, in the thickest part of the thallus. A longitudinal section (fig. 13) of the thallus near the mid-body region shows the length of the sclerotic cells. These conducting cells are sometimes referred to as ventral cells. They are, if everything below the air chambers may be considered ventral; but the lowest cells of the thallus are least suggestive of tracheids, either in form or in function, as figs. 1 and 2 also indicate. A group of ventral cells (fig. 13) is highly magnified in fig. 14.

The potentialities of such cells as the one most prominent in fig. 14 are demonstrated by a regenerative plant. Fig. 15 is a more or less perfect longitudinal section through the anterior region of the thallus, from which there have grown three adventitious proliferations. Such vigorous cells as the one shown in fig. 14 must be the primordia of these innovations. Plants subjected to greenhouse conditions at the close of the growing season regenerated new shoots readily. Fruiting plants, that is, those bearing archegonial heads, failed to continue development. Possibly a quiescent period is necessary at the change of the season, but it seems more probable that the short daylight periods of the late autumn and early winter are insufficient for the maturation of the sporophytes. The relative senility of the cells in the receptacle would seem to account for their failure to resume development with the return of spring. Apparently their vital-

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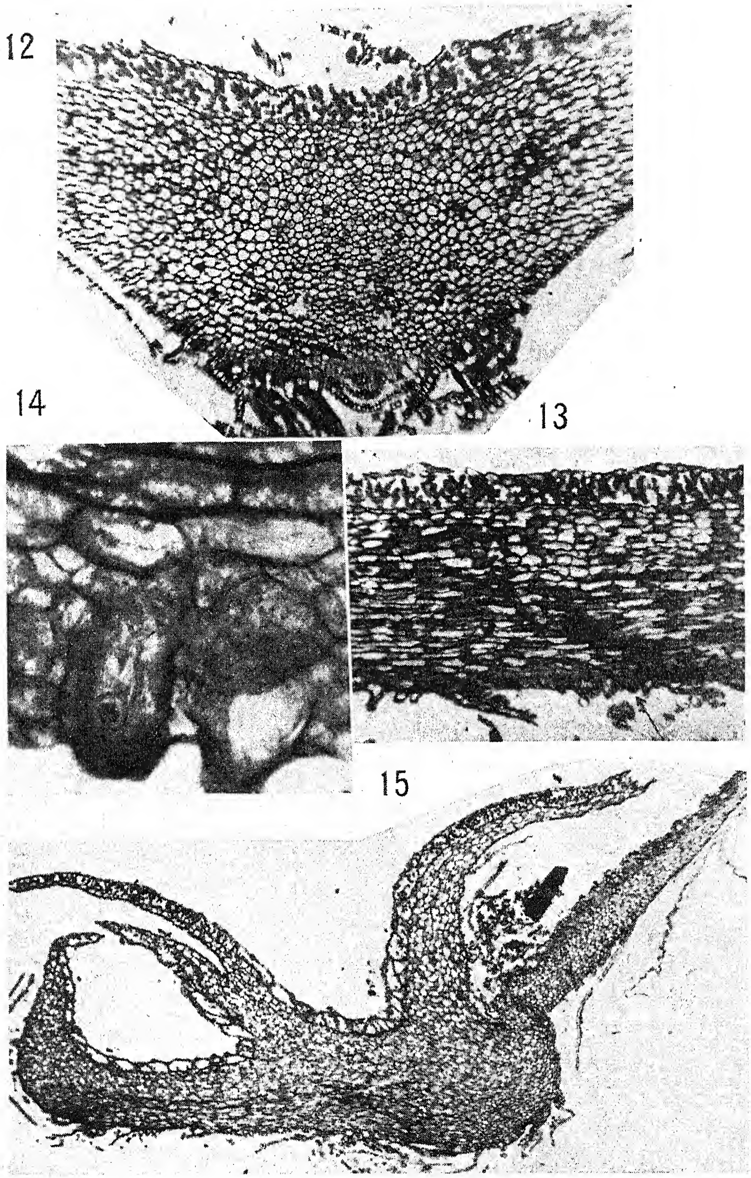

FIGS. 12–15.—Fig. 12, transverse section through midbody region of adult thallus; ×54; fig. 13, longitudinal section of thallus a little lateral to mid-body region; ×66; fig. 14, detail of fig. 13 showing ventral cells; ×535; fig. 15, section of anterior region of thallus showing three regenerative growths; ×33.

ity was sacrificed to the regenerative growths for which the shorter daylight periods are quite adequate.

Summary

1. Twelve to sixteen archegonia are borne in each female receptacle, usually three to four in each quadrant, although as many as six were counted in a single quadrant of a receptacle.

2. Although fertilization is probably 100 per cent, the number of mature sporophytes in a single receptacle varies from one to six, the average number for over 100 heads was 2.98, the dominant number being four sporophytes to each head.

3. The sporogenous cells which metamorphose into elaters seem to be sister cells to the spore mother cells; therefore the number of spores is four times the number of elaters.

4. The number of spores to each capsule was estimated at about 3000; the average number for each head, therefore, would be nearly 9000.

5. The spores, although much less numerous than in *Marchantia*, are decidedly larger. Their diameter is about 75 μ, while the diameter of the spore of *Marchantia polymorpha* is not over 18 μ. The elaters of *Preissia* are smaller than those of *Marchantia*, although their number is greater in proportion to the number of spores.

6. The spores of *Preissia* germinate readily on a solid substrate, and they are viable for a shorter time than the spores of *Marchantia*, as only about 10 per cent of them germinated four or five months after their maturity.

7. The method of spore germination is similar to that in *Marchantia*, in that there is a variety of initial steps, with even greater scope for variation and individuality in *Preissia* than in *Marchantia*.

8. Branching is very common, and in some cases two thalli are initiated from a single spore cell. Early branching is frequent.

9. Unlike *Marchantia*, there are no cells for the storage of essential oils in the young thallus of *Preissia*.

10. The rhizoids of the young thallus, relatively few in number, are of the plain walled type as in *Marchantia*.

11. As there is no definite midrib in the adult thallus of *Preissia*, the thickening in the middle of the young plant is more extensive than is the case in *Marchantia*.

12. The general contour of the advancing young gametophyte of *Preissia* is often quite irregular, since there are so many actively growing points.

13. As in *Marchantia*, a distinct apex with its marginal row of meristematic cells is conspicuous in at least one region of the young thallus.

14. Finally, as in *Marchantia*, there is no single cell functional in the development of the young gametophyte of *Preissia* that may be designated as *the* apical cell.

The writer is indebted to ROBERT G. GUTHRIE for the preparation of the negatives used in the illustrations, and to Sister AUDREY KERBER for collecting some of the plants.

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CURRENT LITERATURE

BOOK REVIEWS

Evolution and classification of cacti

A most interesting little volume has recently been published by BERGER,[1] who has long been a leading authority, not only on the growing but also on the classification of succulents, especially of the cacti. He has been a pioneer in developing our present classification of this difficult group. For many years, as Curator of the Thomas Hanbury Garden at La Mortola, Italy, he grew many of these plants, and had exceptional opportunities to study many species which are rarely found in European gardens.

As is well known to all students of this group, its classification has been very difficult to present in a logical way. This has probably been due to the reluctance of botanists to recognize enough genera. For instance, in his *Species Plantarum*, published in 1753, LINNAEUS reduced all the cacti, some 15 genera, known in his time to the single genus *Cactus*. PHILIP MILLER, botanist as well as gardener, who knew many of these plants living restored the genera *Opuntia*, *Cereus*, and other striking ones, but which those who followed LINNAEUS were slow to accept. Afterward from time to time new genera were described, but these too were reduced by succeeding botanists to synonymy. Then, in 1900, KARL SCHUMANN published his great monograph of the family, but he recognized only 21 genera. A few years afterward OTTO KUNTZE condensed the family into the three genera, *Cactus*, *Pereskia*, and *Pterocactus*, the latter two containing but 14 species, thus leaving almost the entire family (1000 species or more) in the genus *Cactus*, as proposed by LINNAEUS 150 years before!

About 1910 BERGER published one of the most suggestive little papers on the classification of cacti which had yet appeared, and which formed the basis of the classification developed in the monograph of the family published by the Carnegie Institution in 1921–23. BERGER had brought together at La Mortola a large number of cacti, where they had grown luxuriantly, and many had flowered and fruited under his eye. With information obtained from the living plants themselves he prepared this new classification of the genus *Cereus*.

On the origin of the cactus family BERGER has not been able to throw any new light. He agrees with DE CANDOLLE and KARL SCHUMANN that the nearer relationship is with the Aizoaceae. This relationship, however, is most remote. This fact, along with the great diversity in the cactus family itself, seems to suggest a very ancient origin. As the family is wholly American, one would

[1] BERGER, ALWIN, Die Entwicklungslinien der Kakteen. 8vo. pp. iv+105. Jena: Fischer. 1926.

expect to find a closer relationship with some American family than with an Old World one. It is not unlikely that the intergrading family or families do not now exist. Unfortunately geology does not throw any light upon this interesting problem, for no cactus fossil of any kind has ever been found. This is certainly very remarkable, for if the family is so very old, as we are now inclined to believe, some records must have been made in the rocks. Although the cactus plants are made up mostly of water, the spines and hard seeds of many of them might easily be preserved. It has been suggested that the reason these are not found is because the cacti grow in the deserts away from the swamps and lakes. But in many places, such as Lower California and the Islands of the Gulf of California, great cactus plants are found just on the water's edge, which on dying must fall into the sea, while all the streams which flow through these deserts must carry great quantities of cactus débris to the sea.

As to the development in the family itself, BERGER thinks it originated from some *Pereskia*-like ancestor which had normal branches and leaves, and which had a tendency to become succulent, and perhaps without spines. These grew in the tropics on the border of forests. From these main stems were developed two lateral branches, each with a definite type of seed. From one was developed the genus *Cereus* and its relatives (Cereeae), and from the other *Opuntia* and its relatives (Opuntieae).

The classification follows closely that of BRITTON and ROSE. For instance, BERGER divides the family into three tribes, Pereskieae, Opuntieae, and Cereeae. The last and largest is exactly that of BRITTON and ROSE, while in the other two he transfers the genus *Mahuenia* from the second tribe to the first. The genera are the same except that he raises two subgenera of *Opuntia* to generic rank, namely, *Brasilopuntia* and *Consolia*. He also has described as new the genera *Stephanocereus* and *Roseocactus;* he recognizes 129 genera in all. His arrangement of the genera is entirely new and deserves careful study. I have no hesitation in saying that this little book is one of the most important contributions to the study of cacti which has yet appeared.—J. N. ROSE.

NOTES FOR STUDENTS

Taxonomic notes.—EVANS and MEYROWITZ[2] have published a list of the lichens of Connecticut. It is stated that the list includes "records from 79 towns" in the state, and contains 231 species and 65 genera. An interesting contrast is made with the lichen flora of certain other New England states, in the following statement: "Over 700 forms are known from Maine, 375 from Vermont, and nearly 400 from Massachusetts." This contrast probably accounts for the statement that "the study of Connecticut lichens is still in its early stages."

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[2] EVANS, A. W., and MEYROWITZ, ROSE, Catalogue of the lichens of Connecticut. Conn. State Geol. and Nat. Hist. Survey. Bull. 37. pp. 49. 1926.

HUNT[3] has published a list of the Uredinales of New England. The number of species listed is 204, distributed in families as follows: Coleosporiaceae 7, Uredinaceae 34, Aecidiaceae 163. The genera are described, and under each is a list of species with their hosts. Much the largest genus is *Puccinia* with 98 species, and next to it is *Uromyces* with 35 species. Nearly 700 species of host plants are listed, the genera with the largest representation being *Crataegus* and *Carex*.

LEONARD[4] has published a revision of the North American species of *Scutellaria*. This genus is generally distributed in temperate and tropical America. It seems that no full presentation of the group has appeared since its publication by BENTHAM in DE CANDOLLE'S *Prodromus* in 1848. The older names and the specific characters represented a very tangled maze. LEONARD has recognized 62 species, no new species having been found in the United States, but 7 new species from the tropical region of North America are described.—J. M. C.

Essential elements for plants.—The term "the ten essential elements" has attained almost a state of sanctity in plant physiological circles. As SOMMER and LIPMAN[5] point out in their recent paper, the list of ten elements assumed to be the only ones needed for the growth of green plants was, with the exception of the later substitution of iron for silicon, the same as that given by DE SAUSSURE in 1804. Thus for more than a century this concept was held, although ash analyses showed a large number of other elements present in plants. With the improvement in technique in rather recent work, the idea became quite general that, while the ten elements were the only ones necessary in the sense that there could be no growth without them, the presence of other elements improves the growth of plants. These added elements were called by some "stimulants." Still more recently MAZÉ, WARINGTON, BRENCHLEY, MCHARGUE, and others have performed experiments which indicate very strongly that we must add to the list of ten a number of other elements, these being not merely stimulants but absolutely indispensable for plant growth. This literature is reviewed by the authors.

SOMMER and LIPMAN worked with zinc and boron, and instead of showing the need of these elements for one plant, as was true of some of the work of others, they showed that they are needed by several plants of widely separated families, which indicates that they are needed by all green plants. All possible precautions were observed to exclude impurities. Specially purified salts were

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[3] HUNT, W. R., The Uredinales or rusts of Connecticut and the other New England States. Conn. State Geol. and Nat. Hist. Survey. Bull. 36. pp. 198. 1926.

[4] LEONARD, E. C., The North American species of *Scutellaria*. Contrib. U.S. Nat. Herb. **22**:703–748. 1927.

[5] SOMMER, A. L., and LIPMAN, C. B., Evidence on the indispensable nature of zinc and boron for higher green plants. Plant Physiol. **1**:231–249. *figs. 13*. 1926.

used. The plants were grown in a small greenhouse constructed in a larger greenhouse. By screening the ventilators and other means a practically dust-free atmosphere was obtained. Redistilled water was used in making up the culture solutions, and the plants were grown in glass containers made of a kind of glass giving only an insignificant amount of the element being studied to the culture solution. If it was possible to do so, the seed was cut from the seedling soon after germination.

The results are presented in the form of photographs. The contrast between the plants grown with zinc and boron present and those grown without one or the other of these is very striking, and certainly gives strong evidence that these elements are essential for the growth of green plants, and do not serve merely as "stimulants." Considerable other work has been done by the authors on aluminum, silicon, and chlorine, and the results of this will be presented in later papers.

BRENCHLEY and WARINGTON[6] have published a recent paper showing that boron is essential for various plants, including several legumes and the melon. Various cereals and candytuft completed their development in the absence of this element. The question is raised whether there is a class distinction here, or whether the latter plants merely require so little boron that there is enough in the seeds to supply their needs. The latter interpretation would seem to be the correct one, for SOMMER and LIPMAN's work strongly indicates the need of boron by barley. As to the physiological significance of boron, BRENCHLEY and WARINGTON state that in the case of the broad bean their work suggests a connection with the absorption or utilization of calcium. They also performed experiments to determine whether the nature of the substratum on which the plants were grown, the condition of aeration about the roots, the hydrogen-ion concentration, and other features affected the need of certain plants for boron.

Thus it would seem that the term "the ten essential elements" is now obsolete, at least when used in its original sense. The concept, however, still retains the significance that the ten are the elements needed in the largest amounts. But that other elements are indispensable for the growth of plants certainly seems proved by the evidence, and it would seem justifiable to add at the present time zinc, boron, and manganese to the list. Probably other elements will have to be added later, as in fact certain work already indicates.—S. V. EATON.

Investigation of "glacial relics."—TURESSON[7] has made an interesting analysis of the well known *Poa alpina*, by growing side by side plants from various localities of Sweden and Norway, and observing their differences in

[6] BRENCHLEY, WINIFRED E., and WARINGTON, KATHERINE, The rôle of boron in the growth of plants. Ann. Botany 41:167–187. 1927.

[7] TURESSON, GÖTE, Contributions to the genecology of glacial relics. Hereditas 9:81–101. 1927.

structure and in earliness. He finds that the species is composed (in Scandinavia) of three ecotypes: (1) *alpinus*, a dwarf alpine type with high water requirements, early blooming; (2) *subalpinus*, tall with moderate water requirement, widely distributed, especially in the alpine region; (3) *pediacus*, tall, glaucous, with low water requirement, late blooming, restricted to the lowland in isolated localities.

Poa alpina found in lowland situations has usually been considered a relic of the first flora which appeared after the melting of inland ice, but TURESSON thinks that the first plants to follow the retreating glaciers must have been early blooming and resistant to cold. He holds that the present lowland form could not have been evolved from alpine populations by selection of biotypes fitted for lowland conditions, because no such biotypes could exist in the rigorous climate of the time. It is concluded, therefore, that the lowland forms are from other stock and came in during a later and warmer period. *Poa alpina*, like many other species, was subjected in the Ice Age to a great destruction of its low altitude biotypes, and hence has been able to occupy only small and isolated areas in the lowlands. No such depauperization of alpine biotypes took place, so the species is now well represented at high altitudes.

Students of plant geography in this country could probably find answers to some of their problems by determining the ecotypes of their species with TURESSON's method of growing together specimens from different localities and altitudes. It is possible that in America also the "relic hypothesis" has been overworked.—F. RAMALEY.

Influence of light on seed germination.—ELISABETH KOMMERELL[8] has published the results of her investigations on the influence of light of various wave lengths on the germination of seeds. She conducted quantitative experiments, using the seeds of *Lythrum salicaria* and *Nicotiana tabacum*. The seeds were illuminated in a thermostatic chamber by light of various wave lengths obtained from an Osram lamp. The energy of the different portions of the spectrum was made approximately equal by inserting screens consisting of photographic plates blackened to various degrees. The measurements of energy of the incident light were made with a bismuth silver thermopile and a Paschen galvanometer. The time of exposure of the seeds was adjusted to bring out differences in different regions of the spectrum, since with too long exposure these differences were not apparent.

The results indicate that the percentage of germination varied directly with the wave length, and the author concludes that the germination of seeds is a photochemical process following the EINSTEIN Photochemical Equivalence law. This law may be expressed as $N=\frac{Q}{h\nu}$. N is the number of molecules in-

[8] KOMMERELL, ELISABETH, Quantitative Versuche über den Einfluss des Lichtes verschiedener Wellenlängen auf die Keimung von Samen. Jahrb. Wiss. Bot. **66**:461–512. 1927.

volved in the primary photochemical reaction; Q is the energy absorbed from the radiation and used in activating N molecules; while $h\nu$ is the value of the quantum of frequency ν, and is directly proportional to the frequency. It is apparent that with a constant amount of energy absorbed, there will be more molecules transformed in the region of long wave lengths and low frequencies, since there the value of the quantum $h\nu$ is smaller. A complete test of the law requires the determination of N, Q, and ν.

In the case of the germination of seeds the number of molecules involved is unknown, but if (1) the percentage of germination may be taken as a measure of the number of molecules reacting, and (2) the energy absorbed may be considered as equal in all portions of the spectrum when the incident radiation is made equal, then more germination would be expected in the longer wave lengths, and there would be a linear relation between wave length and percentage of germination. However, if the preceding assumptions (1 or 2) do not hold, then the finding of a linear relation would lose the significance attached to it.

Many attempts have been made by photochemists to confirm the EINSTEIN law, and the results to date are not very encouraging, due to the great experimental difficulties and the uncertainty regarding primary and secondary processes, even in relatively simple chemical reactions. Under these circumstances it seems almost hopeless to seek a confirmation of the law in the complex totality of reactions represented by the germination of a seed.—F. WILCOXON.

Intersexualism in Arisaema.—MAEKAWA[9] has discovered in *Arisaema japonica* the occasional appearance of intersexual spadices. The arrangement of flowers on such a spadix is very distinct, the staminate and pistillate flowers not intermingling, but appearing in distinct groups. All of the intersexual spadices in succeeding years were transformed into pistillate spadices. It was noted that the intersexual specimens are rather frequent in plants that were wounded in former years.—J. M. C.

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[9] MAEKAWA, T., On intersexualism in *Arisaema japonica*. Japanese Jour. Bot. 3:205–216. 1927.

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GERMINATION AND EARLY GROWTH OF CORNUS FLORIDA, SAMBUCUS CANADENSIS, AND BERBERIS THUNBERGII

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 374

OPAL HART DAVIS

(WITH FIVE FIGURES)

Introduction

The causes of delayed germination as summed up by CROCKER (7) fall into two groups: (1) those based on embryo characters, and (2) those based on coat characters. The seeds used in the present study all appear to be associated with the first group of causes.

The embryos are in no case immature, as found by IVES (21) in the holly. The seeds of *Cornus florida* seem rather to belong to a category of seeds, of which examples have been studied by DAVIS and ROSE (10) and ECKERSON (12) in *Crataegus mollis*, by HARRINGTON and HITE (20) in the apple, by PACK (33) in *Juniperus*, and by JONES (23) in the sugar maple. In every case a low percentage of germination is reported for untreated seeds, especially with testas removed, while cold treatment over a long period was necessary to overcome dormancy, and bring about a high percentage of germination. These workers found a temperature of 4° or 5° C. optimum for the process of after-ripening. They have reviewed most of the literature bearing on this type of delay.

Seeds of *Sambucus canadensis* and *Berberis thunbergii* seem to belong to a group sensitive to alternation of temperatures. HAR-

RINGTON (19) has given a comprehensive summary of the literature on this group, and has stated the theories in regard to the effect of temperature alternations on germination. MORINAGA (30) also has reported work with several seeds favored by alternation, including *Berberis thunbergii.*

The present investigation was undertaken to learn as much as possible about the factors related to the germination and early growth of *Cornus florida*, and to extend the work of ROSE (38) on *Sambucus canadensis* and of MORINAGA (30) on *Berberis thunbergii.*

Cornus florida

Cornus florida has a range through southern Maine, southern Ontario, southern Michigan, to Missouri, south to Florida, and west to Texas. It is found associated with all beech-sugar maple woods, and often with white or with white and black oak (the mesophytic oak forest). It is quite sun tolerant, as shown by its ability to grow in the open when transplanted, and by its frequent occurrence associated with hazel, sumac, bayberry, cherry, and sassafras, in semi-xerophytic thickets.

The flower buds form in late summer, and the embryo sac passes the winter in the 8-nucleate stage. MORSE (31) states that in 1906 the pollen shedding began May 14, and that in 1907, although the season was later, the shedding period was practically complete by May 27. He did not observe fertilization, but he noted that flower clusters in which there were no fertilized flowers were soon shed, and that large numbers of such heads could be seen under a single tree. The number of fertilized flowers to a head ranged from one to three. The present writer found as high as eight berries to the head in New York, where the trees are unusually plentiful. MORSE does not state whether unfertilized flowers in such heads developed to berries or not.

A large percentage of empty pericarps (100 per cent in seeds collected from solitary trees) was present in collections made in the woods of east central Indiana in 1923. These seeds showed no indications of attacks by fungi or insects, but were large, well formed fruits containing chambers slightly constricted, and one or more diminutive mummified kernels. The only situation giving seeds of

as much as 80–90 per cent seed content was that of a small group of trees on the north side of a dune, about one-fourth of a mile from Lake Michigan, between it and Long Lake, Indiana. Several other trees on the dunes, sometimes in groups of two or three and sometimes as individuals, showed the ravages of squirrels and birds, which are quite fond of the fruits. The seeds which were on the ground had nearly all been gnawed. Those still on the trees gave 15–20 per cent seed content.

During 1924–25 collections of *Cornus florida* seeds were made in a region noted for the abundance of these trees. During October more than a bushel of fruit was collected from trees on the hillsides east of the Hudson River, around Yonkers, New York. In only one case did the number of empty seeds reach more than 6–10 per cent. The trees often occur in clumps, but the seeds of those rather widely separated were good except in the one case, where the chambers up to 50 per cent were filled with soft milky kernels. The reason for such poor seeds has not been determined; in some cases it may be due to failure of fertilization, and in others to lack of nutrition.

By November 3 the squirrels and birds had cleared the fruit from most of the trees on the golf links and estates, and very few of the seeds could be found under the trees; however, in places less favorable for squirrels a great many of the seeds lay on the ground throughout the winter. It is evident that the attractiveness of their fruits and seeds to birds, and especially to squirrels and other rodents, and the tendency to produce hollow seeds where the trees are isolated, are limiting factors in the spread of *Cornus florida*. The chief determining factors, however, are probably related to the early dormancy of the seed.

Preliminary study.—During 1923–24, three lots of seeds were used in germination studies. The first was collected at Long Lake, Indiana, on October 8. These seeds were allowed to dry in the fleshy berry and were then stored at room temperature. The second lot was collected at Johns Hopkins University during October, and the seeds were dried and stored at room temperature, after the fleshy part had been washed entirely away. The third lot was secured from Conyers B. Fleu at Philadelphia, February 27. These

had been collected from the trees in October, and stored in the berry at 30° F. from that time until they were sent.

For 1924–25, seeds were collected at Yonkers, New York, during October, and from one tree December 24. Five pounds of seeds which had been stored air-dry in the pulp, and one-fourth pound which had been stored moist in the berry at 50° C. until February 28 were obtained from CONYERS B. FLEU.

The seeds of the various lots differed very little from one another in size, color, or other points. The fruit of *Cornus florida* is an elliptical drupe, scarlet in color, the fleshy part of which somewhat resembles the red haw in texture. The flowers are epigynous, the sepals remaining on the apex of the fruit. The stone consists of a hard woody layer; an inner coat loose from the seed (and usually partially adhering to the woody layer, so that it is never left intact when the stones are broken); a grayish, flaky membrane of cells crushed together; the outer layer of the endosperm (a single layer of thick-walled cells); the endosperm; and the embryo lying exactly at its center and extending the full length of the seed (fig. 1). The second layer resembles quite closely the red testa of the peanut, being golden brown in color without, and gray within.

The outer layer of the endosperm forms a tough whitish membrane, which expands as the cotyledons grow and the stored material of the endosperm is used. If the seedling is kept in a moist atmosphere this coat slips off the cotyledons when they pull erect from the seed, or as they reach a size of 0.5×1 cm. If it remains on the cotyledons long enough to dry, however, they show some difficulty in escaping from it. It is very gradually split along the edges, and the cotyledons separate, with the membrane still clinging to them.

The embryo consists of the two cotyledons as broad as the seed (2.5–3 mm.) and about 4 mm. long, and a hypocotyl which from later development seems to be of two parts, a 0.5 mm. area forming the root and a 2 mm. region elongating and greening to form the stem. A rounded mass of meristematic tissue indicates the place of origin of the plumule. MORSE (31) found the young embryo well differentiated into cotyledons, hypocotyl, root tip, root cap, and embryonic tissues as early as July 28. Cross-sections of seeds col-

lected in October show the cotyledons to be differentiated into upper epidermis, two rows of palisades, parenchyma, and lower epidermis, with rounded clusters of undifferentiated vascular tissue. The

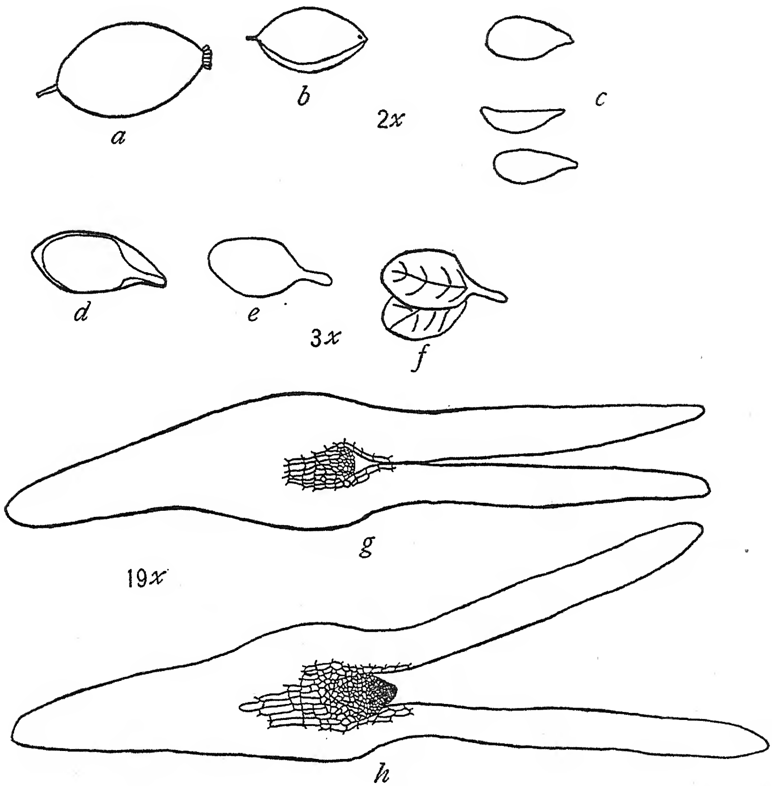

FIG. 1.—Fruit, seed, and embryo of *Cornus florida: a*, fruit; *c*, seed with stony covering removed; *b*, stone; *d, e, f*, embryo; *g*, plumule of air-dry seed; *h*, plumule of germinating seed.

hypocotyl portion shows elongated cells in the protostelar region, which differentiate into xylem and phloem immediately on the elongation of the hypocotyl.

Preliminary tests of hygroscopic moisture showed that the air-dry seeds range from 2.8 to 6 per cent. The reduction of the moisture content to this rather low amount had no effect on later germination, perhaps because the seeds are of the fat-protein type, having a

ipoid content of 48 per cent. Neither the bony covering nor testa immediately beneath it plays any part in causing the delay, except in so far as they act as a mechanical restraint to feebly swelling seeds. The water intake of seeds in the pericarp in distilled water reached as much as 61 per cent in three days, while that of seeds taken from the coats reached 50.5 per cent in twelve hours, at which time disintegration became sufficiently marked to interfere with the results. Large numbers of seeds freed from the pericarp and testa, and subjected to low temperatures for varying periods required the same length of treatment for inducing germination as those seeds tested with pericarps intact. Treatments with ether and ethylene were unsuccessful, as were also those in which an atmosphere high in O_2 was used.

For sterilizing the seeds used in these preliminary tests, Javel water was used with moderate success. It was made according to directions by MOLISCH (29) and diluted to 10 or 20 per cent. The 10 per cent was found the more satisfactory for disinfecting the seeds of *Cornus florida*. The seeds were sterilized with this solution according to the discontinuous method of DUGGAR and DAVIS (11).

METHODS

For germination studies during 1924–25, seeds were washed free from the pulp, sterilized for one-half hour in 0.25 per cent uspulun,[1] washed carefully with boiled tap water, and put under germinative conditions. To remove the pulp the seeds were run through an ordinary food chopper with the small cutting knife removed. The stones were then washed entirely free from pulpy material, sometimes by the aid of quartz sand rubbed with them in a canvas bag. All empty seeds floated and were eliminated.

Seeds thus prepared were placed under the conditions indicated in tables I to V. Large enough quantities (5000–10,000 seeds) were put under each germinative condition to permit physiological and chemical studies during the process of after-ripening and the early stages of germination. Seeds of this lot stored air-dry and a five pound lot of seeds stored dry in the pulp were also used for germination and other studies.

[1] Uspulun is a seed disinfectant containing hydroxymercurichlorophenol sulphate prepared by the Bayer Company.

Catalase activity was determined at intervals of ten to fourteen days during the period of after-ripening. The method has been described by APPLEMAN (2), CROCKER and HARRINGTON (8), RHINE (37), and several other workers.

Microchemical tests were made on air-dry seeds, on seeds which had been fairly well after-ripened at low temperatures, on those which had swollen sufficiently to split the pericarp, and on some which had begun elongation of the hypocotyl. Sections were cut freehand, and tests as suggested by ECKERSON (13) were applied.

Macrochemical analyses were made on samples of seeds from the same stages as those studied microchemically. KOCH'S (26) method was followed rather closely, but with some modifications suggested by Dr. H. R. KRAYBILL. Since the dogwood seeds are quite small, and tend to change in weight and color on being ground, they were put into the samples intact. The alcohol was evaporated from the total extract, leaving 100–150 cc. of solution. Separation was then made by shaking the solution with ether in a separatory funnel. The sugar content was determined by the QUISUMBING-THOMAS (36) method, with invertase as the hydrolytic agent for sucrose.

Ammonia and amid nitrogen were determined by distillation under reduced pressure. The apparatus described by VAN SLYKE (42) was used. For amino-nitrogen the Van Slyke micro-apparatus was used, and the calculations made on the basis of the table in MATHEWS (28). The phosphorus content of the lipoid fraction was determined by the Neumann-Pemberton method and the iodine number by the Hanus method, as described in the Official Methods (1).

Studies of respiration were made continuously over a period of 58 days on lots of fifteen seeds each, kept at 6°, 12.5°, and 22° C. respectively, and were then repeated in duplicate on lots of twenty at 2.5–7°, 10–14°, and 20–22° C. respectively for a period of 112 days, followed by eleven days at room temperature (18–26° C.) to permit germination of those seeds which were after-ripened.

For these studies a modification of the apparatus devised by OTA (32) was used. Instead of being forced through, CO_2-free air was drawn by suction through the apparatus. As seen from fig. 2, the chief modifications consisted of a train of wash bottles, two con-

taining saturated NaOH and the one next to the apparatus containing saturated $Ba(OH)_2$, and a trap bottle beneath the apparatus to which suction might be applied and into which the normal NaOH used to absorb the CO_2 of respiration might be drawn. A mercury manometer was substituted for the water manometer, the seed chamber being calibrated in order that the O_2 absorption could be calculated roughly from the change in pressure.

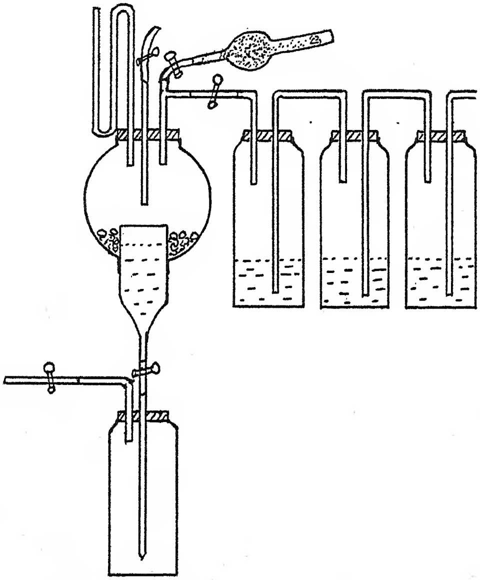

FIG. 2.—Apparatus used for respiration studies

At intervals the manometer reading was taken, along with a record of temperature and barometric pressure. The cup was drained, rinsed with distilled water, and redrained by suction continued long enough to draw out any unabsorbed CO_2, and the apparatus reset as before. The NaOH was titrated as described by BROWN and ESCOMBE (4).

RESULTS

GERMINATION.—When a single seed or lot of seeds of *Cornus florida* is completely after-ripened, a sudden imbibition of water and swelling sufficient to split the pericarp occurs in 6–10 hours at 20° C.,

after which elongation of the hypocotyl becomes apparent in another 6–10 hours. Only occasional freshly gathered seeds swell sufficiently to break the bony layer, after which very slow elongation occurs.

Seeds with the bony layer removed sometimes show abnormally slow elongation of the hypocotyls up to 1 or 2 per cent in either light or darkness. In the light the cotyledons often become green, beginning at the edges where the endosperm layer is absent or extremely thin, then expand, splitting the membrane, and are gradually pushed from the seed by the elongation of that region of the hypocotyl immediately below, which is structurally stem. Such abnormal growth may be followed by a very slow elongation of the hypocotyl, gradually becoming normal in rate, or the hypocotyl may finally be slipped from the parted endosperm by the weight of the expanding upper part of the seedling. In the latter case the hypocotyl may grow or the decomposition of the endosperm may have progressed so far before its release as to kill the delicate tip cells and prevent normal root growth.

Normally, as in seeds with the bony covering intact, the cotyledons seem to act as haustoria for the seedling, while exhausting the stored material of the endosperm. When well expanded, the seedling, like that of many dicotyledons, pulls erect, leaving all seed coverings behind in the soil. The cotyledons then grow and function as true leaves until the third or fourth pair has appeared.

Seeds, whether freshly gathered or those subjected to cold treatment, are not at all uniform as to the depth of their dormancy. However, except for the occasional seed just mentioned, no germination occurred until the seventy-fifth day of cold treatment, and then only from a lot at 10° C.

Of seeds from 0°, 5°, 10°, 15° C., taken from the bottles in duplicates of twenty at intervals of two weeks and put at 15°, 20°, 24°, and 27° C. respectively in petri dishes, the highest percentage germinated from the 0° or 5° lots transferred to 15° C. However, germination at 15° of such partially after-ripened seeds extended over 30 days, while at higher temperatures it was complete in 10 days. The difference in percentage of germination at the different higher temperatures decreased as the length of the cold storage period in-

creased. The tendency of higher temperatures to induce secondary dormancy in incompletely after-ripened seeds decreases with a lowering of the temperature; and though after-ripening does not occur at 15°, the continuance of this process when near completion seems to be favored by that temperature.

Tables I and II show that after-ripening takes place most quickly at 10° in the large petri dishes where there is a considerable volume of air, while in the sand bottle there is such a fall in the percentage at 10° that the 5° temperature becomes most favorable. There usually appeared to be about a ten day to two week interval between the germination of seeds at 10° and 5°, and again between 5° and 0° C.

The petri dish with moist cotton and filter paper seed bed seems to provide sufficient air and moisture for optimum germinative conditions, but only a few of the seeds may be after-ripened at a time in such a container. In addition, the petri dish is rather difficult to keep sterile for a period of 130 days or more.

The moist quartz sand mixture must be aired, and moistened as frequently as every two or three days in a dry atmosphere. Otherwise the water tends to settle, leaving the upper layers dry, and causing a lack of uniformity in after-ripening. In addition there seems to be a lack of air for the seeds at 10° as they approach completion of after-ripening, but with a fairly humid atmosphere at 5°, the sand bottle is quite efficient for quantity production of after-ripened seed. There is little difficulty with molds.

Peat has proven so far the most satisfactory medium for germination, but has been used less extensively than the others. As suggested by HARRINGTON (17) for canton flannel, it has the advantage of coming in closer contact with the large seeds without interfering with the air supply. It also maintains a more uniform moisture supply. ECKERSON (12) has reported that dilute acid favors after-ripening of *Crataegus mollis*. The pH value of peat is about 4.5, but it is doubtful whether the reaction value is an important factor, since dilute HCl, H_2SO_4, butyric acid, and acetic acid in the concentrations recommended as most favorable by ROSE, appeared to have no effect, and nearly as good percentages of germination were obtained with the other seed beds without acid conditions when the moisture was kept constant. The bottle at 5° mentioned in table I required no airing or moistening during the 112 days, and would probably

TABLE I

AFTER-RIPENING AND GERMINATION UNDER VARIOUS CONDITIONS OF SEED BED AND TEMPERATURE

Storage temperature (° C.)	First germination: Days to	First germination: Percentage	Germinative condition	No. of seeds	Total days	Total percentage
15	....	....	Petri dish	50	160	0
			Sand bottle	100	160	0
			Peat bottle	200	160	0
10–16	....	....	Sand bottle	900	160	23
10	107	40.0	Petri dish	50	148	86.0
		28.0	Petri dish	50	148	74.0
		15.0	Sand bottle	100	148	51.0
		32.0	Sand bottle	100	148	53.0
		34.8	Sand bottle	500	179	65.6
5	107	4.0	Petri dish	50	179	74.0
		8.0	Petri dish	50	179	68.0
		13.0	Sand bottle	100	179	78.0
		21.0	Sand bottle	100	179	80.0
		13.6	Sand bottle	500	179	76.0
		......	Sand bottle	900	82	15.0
		......	Sand bottle	900	110	45.0
		......	Peat bottle	200	112	82.0
0	....	....	Sand bottle	900	87	21.5
			Sand bottle	900	100	26.0
			Sand bottle	900	113	56.0
			Sand bottle	900	147	78.1
			Sand bottle	900	147	76.0

TABLE II

AFTER-RIPENING AND GERMINATION IN LARGE PETRI DISHES AT LOW TEMPERATURES

Temperature (° C.)	No. of seeds	Percentage germination after: 107 days	131 days	140 days	Total percentage	Total days
5– 8	150	0.........	44.7	54.7	74.0	160
		0.........	38.0	58.7	76.7	160
		0.........	52.7	60.0	80.0	160
		0.........	6.0	67.3	91.7	190
		0.........	0.0	0.0	86.0	190
10–13	150	17.3.......	56.0	....	97.3	150
		7.3.......	50.0	....	95.3	150
		0.0.......	60.7	....	76.7	140
		14.0.......	90.0	....	97.0	140
		8.0.......	79.3	....	82.7	134
		14.3.......	88.7	....	91.3	134
		14.3.......	83.3	....	91.7	138

have given a higher germination percentage if left longer at this temperature. All of the materials used require sterilization in a steam autoclave for 30–45 minutes at 15 pounds pressure to insure moderate freedom from infection during the long period of after-ripening.

TABLE III

GERMINATION OF CORNUS FLORIDA SEEDS TRANSFERRED FROM HIGHER TO LOW TEMPERATURE AFTER COMPLETE DROP OF CATALASE

Container	Higher temperature		Lower temperature		Percentage germination
	° C.	Days	° C.	Days	
Petri dish....	24	51	5	130	88
Petri dish....	27	51	5	130	93*
Bottles.......	10	158	5	74	83
Bottles.......	15	83	5	159	67
Bottles.......	24	96	5	136	86

* Larger percentage here due to less infection.

Table III shows the recovery of seeds which had been put under germinative conditions at temperatures unfavorable for after-ripening. Other seeds were permitted to lie dormant in the soil in flats for more than 130 days, then were placed out of doors in early March. During the month and a half of exposure to temperatures near freezing, almost a fourth of the seeds after-ripened, germinating as soon as the temperature rose.

TABLE IV

GERMINATION OF SEEDS PLANTED IN COLD FRAMES NOVEMBER 28 AND LEFT OVER WINTER

No. of seeds	Condition during winter	Approximate winter temperature	Date of mulch removal	Percentage germination
1000.........	Unmulched	Ground frozen*	....	51.1
1000.........	3 inch mulch	3° C.	April 1 and April 12	72.2
1000.........	6 inch mulch	3–5° C.	April 1 and April 12	77.3

* Minimum atmospheric temperature during winter −3° F.

The difference in germination of mulched and unmulched seeds, as shown in table IV, may be explained on the basis of individual variability. Germination in the storage bottles at low temperatures continues over a period of 45–50 days. Air-dry seeds (33) or seeds in

the pulp are uninjured by freezing, but freezing wet seeds, which had been in moist sand at 0° C. two weeks and had reached a moisture content of 20–30 per cent, produced 30–40 per cent dead seeds, even when the freezing lasted only a few hours and was so light as merely to make the sand cling together in the bottles. A still briefer exposure to freezing proved completely fatal among seeds which had reached the final stage of after-ripening, and had broken their bony covering. Seeds, then, which reached this stage during warmer days of January or February, were killed by subsequent cold weather. Seeds in the mulched beds were not subjected to freezing. Several cracked seeds, with kernels decayed, were found on the bare ground under trees in March and April.

The mulched beds contained several seeds which did not completely after-ripen in a single winter, and also some injured ones, for quite often there are slight insect or fungus injuries in the kernels of freshly collected seeds.

The percentage of killing as shown by table V was much higher for seeds overwintering in moist sand than for those covered with an inch of soil. One would expect the soil temperature to remain higher than that of sand, which has no organic matter. Since the bottles in the sandpit were all inclined mouth downward at an angle of 15°, there was no evaporation from the tops, and the moisture remained quite uniform throughout.

The gradient of decreasing soil moisture content was from the greatest toward the least depth, but the differences were insufficient to affect the results to any marked degree. The atmospheric temperature when the seeds were removed from the pit was still quite variable, falling to freezing or below much of the time. That probably accounts for the fact that the highest percentage of germination was obtained from seeds at the greatest depth, for there the minimum temperature was higher. The final differences in germination between the two more deeply buried lots was probably due to the individual variability of the seeds composing them.

Storing seeds intact in the pulp does not appear successful under any condition. Seeds which had been stored moist in the pulp in rather open containers at 30° F. from October until the last of February, showed 30–40 per cent infection with *Fusarium*, *Penicilli-*

um, and *Aspergillus*, while the same seeds had reached 60–70 per cent infection by the end of March. Of seeds stored similarly at 50° F. until the end of February, 100 per cent showed bacterial infection, in some cases accompanied by *Penicillium* and *Aspergillus*. Seeds sterilized with pulp intact, stored at low temperature in sand bottles, and those buried at greater depths were infected chiefly with bacteria, but those buried near the surface contained in addition a black soil fungus.

Observations and collections made in the woods during the spring and summer proved that when *Cornus florida* seeds had lain under leaves in a moist situation during the winter, they germinated as

TABLE V

GERMINATION OF SEEDS BURIED IN SAND BOTTLES OCTOBER 21

No. of seeds	Depth in inches	Condition	Percentage germination while buried	Days buried	Total percentage germination	Days after removal
200......	1–3	Free of pulp	8.5	176	13–	14
200......	4½–6½		54.1	176	91.8–	14
200......	10½–12		64.0	176	88.5	14
100......	1–3	Sterilized in pulp	0.0	176	0.0	30
100......	4½–6½		0.0	176	0.0	30
100......	10½–12		0.0	176	0.0	30

early as the middle of April and came up through the leaves. Seeds in less moist situations among the leaves required cold treatment before germination, and were in some cases still in the scarlet fruit. Seeds under trees where the ground was nearly bare were frequently all gnawed open by the squirrels, but even where unmolested were either cracked and decayed or required a period of low temperature. No seedling of a few years' growth could be seen where the natural condition of the ground seemed to be that of comparative lack of covering of any sort.

At a nursery on Long Island a dogwood tree was growing in the midst of a field of rhododendrons. During the winter the ground had been mulched. In August large numbers of young dogwood seedlings grew vigorously around the tree over a radius of several feet.

CATALASE.—The results of a preliminary test as to the effect on catalase activity of soaking the ground seed material appear in

fig. 3. The temperature chosen as most favorable for soaking without the introduction of the effects of bacterial action was 15°. There was no apparent change in the catalase until about the tenth hour.

The results shown in the tables, with the exception of those in table VI, were calculated to 0° C., and 760 mm. pressure for 0.1 gm. wet weight, which is the equivalent of 0.06 dry weight. The results represent the average for three to five runs. As may be seen from table VI, the catalase content of a lot of seeds, as measured by O_2 liberated, may not be at all proportional to their weight. The catalase content seems rather to depend on the condition of the seeds.

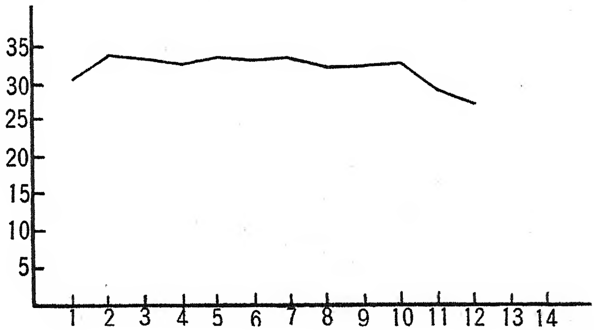

FIG. 3.—Effect on catalase activity of soaking ground material: vertical axes represent cc. O_2 liberated in 10 minutes per 0.5 gm. air-dry weight; horizontal axes represent hours of soaking.

Table VII and fig. 4 show the effect on catalase activity of *Cornus florida* seeds of storage at different temperatures under germinative conditions. Catalase activity and germinability are closely correlated in these seeds. Both show great individual variation; both increase under storage at low temperatures; and both are decreased by higher temperatures, germination being inhibited where the catalase drops below that for air-dry seeds. A similar drop in catalase accompanied by reduced vigor in germination has been reported by SHULL and DAVIS (40) for non-germinating *Xanthium* uppers with coats intact, kept in the germinator for a long period. DAVIS (9) and others, working in the laboratories at the Boyce Thompson Institute, have noted the same drop in catalase, but in these latter instances it is associated with a deepening of original dormancy or a

secondary dormancy, such as is suggested by table VIII for *Cornus florida*. Germination had begun by the eighty-fifth day in the lots at 0°, 5°, 7°, and 10° C., but unsplit seeds were selected for catalase determinations. All the seeds used were taken from the sand bottles, in which, as noted, there was a drop in germination at 10° C. Seeds which had dropped extremely low in their catalase content were slow at first in resuming it at low temperatures, as indicated in

TABLE VI

WEIGHT VS. INDIVIDUAL VARIATION AS FACTORS RELATED TO CATALASE ACTIVITY IN AIR-DRY CORNUS SEEDS

No. of seeds	Grams	Cc. O_2 liberated at 25° C. in		
		2 minutes	5 minutes	10 minutes
6	0.0798	33.3	42.8	49.9
	0.0748	29.4	38.2	46.2
	0.0787	35.7	41.6	53.8
	0.0699	34.0	43.2	50.8
	0.0763	29.6	37.7	45.0
4	0.0543	27.4	35.3	43.6
	0.0608	20.3	27.0	35.1
	0.0468	22.5	29.2	36.6
	0.0445	25.4	33.0	40.6
2	0.0293	12.6	16.8	21.2
	0.0254	12.8	16.0	20.4
	0.0335	9.4	12.6	15.0
	0.0250	11.1	14.4	17.7
	0.0259	13.8	18.4	22.6

table IX, but their recovery is complete as gaged by either catalase or germinative behavior.

DAVIS (9) has devised a test by which living and dead seeds may be distinguished on the basis of catalase content before and after soaking. The catalase content of dead seeds falls markedly on soaking, while that of living seeds remains nearly constant. The results of such a test applied to seeds of *Cornus florida* may be seen in table X. The catalase activity had in the beginning increased most noticeably in the lots of seeds at an alternation between freezing and 5° C.; however, after the second freeze and thaw the seeds were all dead. The catalase remained approximately what it had been at the time of kill during the two or three days the seeds were

left at 5° C. In less than 24 hours after their removal to room temperature bacterial action and disintegration had taken place in every seed. Seeds which had been stored at 15° prior to killing by freezing retained the much lower catalase content they had shown before freezing, but lost nearly half of that on soaking. The amount

TABLE VII

EFFECT OF TEMPERATURE ON CATALASE ACTIVITY OF SEEDS STORED UNDER GERMINATIVE CONDITIONS

Days stored	Cc. O_2 liberated per unit dry weight in 10 minutes at different temperatures (C.)									
	0°	5°	7°*	10°	12°*	15°	17°*	20°	24°	27°
0	43	43	43	43	43	43	43	.....	.....	.....
25	43	44	53	41	46	41	37	.....	.....	.....
37	38	48	41	61	35	44	25	.....	.....	.....
56	43	53	39	47	34	34	20	.....	.....	.....
65	37	.....	41	.....	28	16	.....	.....	.....	.....
74	43	72	47	47	29	34	19	.....	.....	.....
83	47	.....	47	.....	29	.....	19	.....	.....	.....
95	59	58	64	41	38	25	17	.....	.....	.....
130	.....	99	.....	62	.....	26	.....	16	15	10
Split seeds	90–121 cc.	.....	0.5 on radicle-169 cc.							

* Fluctuating but approximately as given.

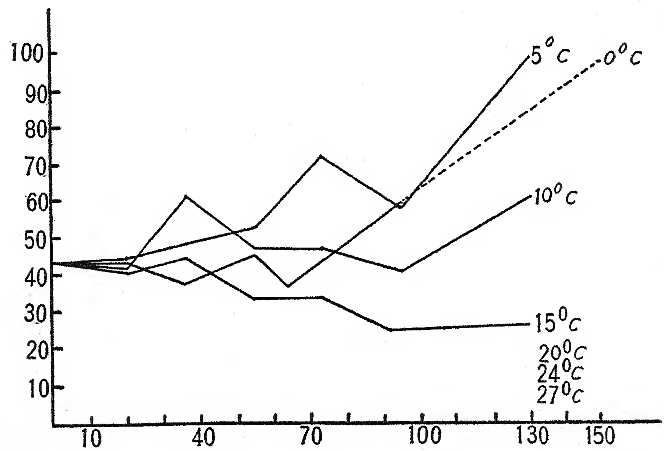

FIG. 4.—Effect of different temperatures under germinative conditions on catalase activity of seeds: vertical axes represent cc. of O_2 liberated in 10 minutes per unit of dry weight; horizontal axes represent days.

of drop seems to increase with a rise in soaking temperature, but in every case for *Cornus florida* seeds there is a noticeable drop for

TABLE VIII

EFFECT ON CATALASE ACTIVITY OF TRANSFER FROM LOW TO HIGHER TEMPERATURES BEFORE COMPLETION OF AFTER-RIPENING

Low temperature		High temperature		Cc. O_2 liberated in		
° C.	Days	°C.	Days	2 minutes	5 minutes	10 minutes
	....	20	95	6.6	11.3	14.1
15..........	53	20	43	7.3	13.5	17.0
10..........	53	20	43	11.8	20.4	26.4
5..........	53	20	43	14.0	23.9	30.2
0..........	53	20	43	11.0	20.0	26.2
	....	24	95	....	8.8	11.4
5..........	53	24	43	14.0	19.4	23.3
0..........	53	24	43	7.7	13.8	18.4
10..........	53	27	11	5.1	8.2	11.2
5..........	53	27	11	7.0	12.0	16.0
0..........	53	27	11	9.1	14.0	17.4

TABLE IX

EFFECT OF COLD TREATMENT FOLLOWING HIGH TEMPERATURES UNDER GERMINATIVE CONDITIONS

High temperature		Low temperature		Cc. O_2 liberated in		
°C.	Days	°C.	Days	2 minutes	5 minutes	10 minutes
15	83	5	12........	7.3	14.2	19.0
			26........	7.0	12.4	15.6
			42........	9.0	20.7	25.3
			62........	14.5	....	31.8
			82........	22.5	37.0	45.0
			159........	68% had germinated at 5° C.		
24	101	5	8........	3.6	7.0	9.0
			24........	6.2	12.2	16.2
			44........	7.0	13.6	18.0
			64........	11.0	19.6	24.0
			136........	86% had germinated at 5° C.		

soaked dead seeds, even when the original content is quite low. For living seeds soaked intact, the catalase remains constant or in most cases increases slightly.

MICROCHEMICAL TESTS.—The results of microchemical tests appear in table XI. *Cornus florida* seeds have a high protein content, occurring as aleurone grains packed closely in the cells and surrounded by a fat which squeezes out of the cut surface and forms large drops over and around the sections. After the imbibition of water into the seed, these grains become irregular in shape, and finally break down in the embryo and adjoining endosperm as swelling occurs. Color reactions indicate the presence of protein materials and hydrolytic products where the aleurone grains have disappeared. The biuret reaction has changed from bluish to pinkish lavender at the close of after-ripening, and an amino acid which has

TABLE X

EFFECT OF SOAKING INTACT SEED ON CATALASE ACTIVITY OF LIVING AND DEAD SEEDS

DESCRIPTION OF MATERIAL	Cc. O_2 LIBERATED IN 10 MINUTES FROM			
	Soaked	Temperature (C.)	Hours	Unsoaked
Killed by freezing..........	20.6	15	24	33
	19.6	20	24	....
	17.3	32	24	....
Air-dry, living.............	37.2	15	24	35.5
5° C. stored 83 days........	43.3	15	36	40.0

not been identified crystallizes out on the addition of absolute alcohol.

Air-dry seeds in a great many cases show no trace of starch, but some may contain a considerable amount, as indicated in fig. 5, which shows the starch content at later stages as well. As after-ripening progresses the starch content increases, until the hypocotyl, lower part of the cotyledons, that part of the endosperm adjoining the hypocotyl, and the two outer layers of endosperm cells over the rest of the seed become well packed with grains. In the third layer of the endosperm occasional smaller grains appear in active movement. At this stage the seed is likely to germinate quickly on removal to room temperature. The starch grains then disappear, beginning at the hypocotyl tip. Seeds at 10° presented in some cases on the forty-fourth day the same apparent starch content as those

at 5° for 74 days, but other seeds at 5° showed a content equal to that of completely after-ripened seeds. Acidity conditions were difficult to ascertain by colorimetric methods, since there was adsorption of the dye, for cresol purple, at the critical point. However, the cell contents aside from the adsorbing proteins appeared to remain about 6.2–6.4 throughout the period of after-ripening. Other changes may best be discussed under macrochemical results,

TABLE XI

MICROCHEMICAL TESTS ON SEEDS OF CORNUS FLORIDA

SUBSTANCE	FORM	TEST	AIR-DRY	AFTER-RIPENED	SWOLLEN TO SPLIT PERICARP
Lipoid	Matrix	Sudan	+++++	+++++	+++++
Protein	Aleurone	Xanthoproteic I. K. I Millon's	+++++ +++++ +++++	+++ +++ +++	Gone from hypocotyl tip and surrounding endosperm
	Soluble	Same as preceding Biuret	+ Blue-lavender	+++ Pink-lavender	+++++ Pink-lavender
Amino acid	....	Crystallization	....	?	++++
Sugar	Reducing	Flückigen Phenylhydrazine osozones Methyl phenylhydrazine osozones	Trace Trace —	Trace Trace —	Trace Trace —
	Sucrose	Hydrolysis flückigen Phenylhydrazine osozones	+++ ++	++++ ++++	+++ +++
Phytosterol	....	H_2SO_4	+	+	+
Acidity	....	Indicator series	6.0–6.4	6.0–6.4	6.0–6.4
Pectic lamellae	....	Methylene blue Ruthenium red	+ +	+ +	+ +
Cellulose	....	Polarization Hydrocellulose	+ +	+ +	+ +
Starch	Localized	Iodine Polarization	− to + − to +	+++++ +++++	+++ +++

since the microchemical tests were preliminary to quantitative determinations.

MACROCHEMICAL RESULTS.—The results of chemical analyses of *Cornus florida* seed appear in table XII. PACK (34) obtained similar results in many cases for *Juniperus* during after-ripening and early seedling growth. He has discussed quite fully the probable chemical and physiological interpretations of his results. However, the seedling stage he has considered is somewhat further advanced than the stage studied for *C. florida*.

Similarly, MILLER (27) for the sunflower, and GREEN (15, 16) for *Ricinus* have studied germination well advanced in oily seeds, while CHOATE (5), JODIDI (22), and FODOR and REIFENBERG (14) have discussed protein changes at similar stages in wheat, corn, and peas. Their results of course show more pronounced differences as to fats and protein between the air-dry seeds and the seedling conditions.

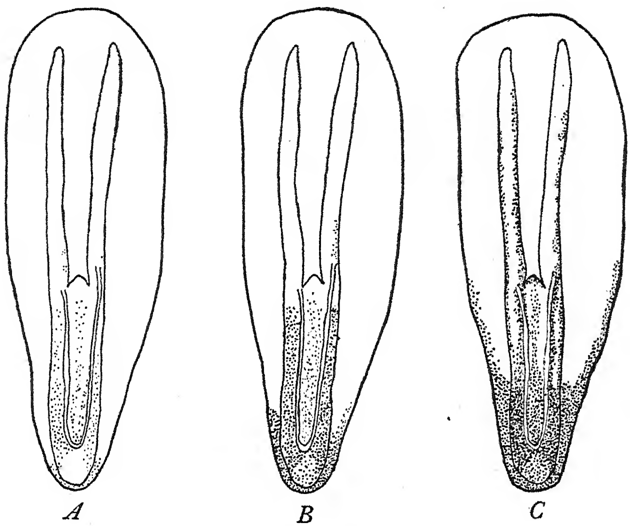

FIG. 5.—Starch accumulation during after-ripening of seeds of *Cornus florida:* *A*, air-dry (maximum); *B*, 42 days at 5° C. (maximum); *C*, 44 days at 10° (maximum); 74 days at 5° (minimum).

The general direction of change is indicated first by an accumulation of active, readily utilized materials such as sucrose, starch (localized in the hypocotyl and adjoining endosperm), amino acids, and soluble proteins during the after-ripening period, and the utilization of part of these following the intake of water as germination begins, accompanied by further breakdown or hydrolysis of the more stable fats and proteins. One might consider the increase of sucrose with increased osmotic activity as perhaps the chief factor in the swelling process which initiates germination. The 2 per cent increase does not represent the total difference between the

sugar content of an air-dry seed at the earliest stage and one completely after-ripened, but rather the difference between the average

TABLE XII

RESULTS OF MACROCHEMICAL DETERMINATIONS CARRIED OUT ON SEEDS OF CORNUS FLORIDA

	AIR-DRY		AFTER-RIPENED		SWOLLEN TO SPLIT CARPELS AND SOME GERMINATED	
	a	b	a	b	a	b
Total gm. dry weight......	18.84	18.84	19.9386	....	27.6742	....
Percentage moisture.......	5.8	5.8	42.7	....	53.8	....
Total F_3 in gm...........	7.348	7.342	7.466	....	10.423	....
Total as percentage dry weight...............	39.0	39.45	37.45	....	37.7	....
Total solids F_2 in gm......	2.295	2.363	3.526	....	4.017	....
Total as percentage dry weight...............	12.2	12.5	17.7	....	14.5	....
Total F_1 gm.............	9.197	9.047	8.947	....	13.234	....
Total as percentage dry weight...............	48.8	48.0	45.0	....	48.5	....
Total nitrogen in F_1.......	0.0	0.0	0.0	....	0.0	....
Phosphorus of F_1 as percentage of F_1.............	0.124	0.119	0.142	0.118	0.132	0.121
Phosphatides as percentage of dry weight..........	1.57	1.47	1.65	1.37	1.65	1.51
Saponification number....	283.0	....	....	....	....	....
Iodine number, dry weight	137.0	139.0	137.0	139.0	136.0	126.0
Free fatty acid as cc. N/10 per 100 gm...........	47.0	....	....	....	48.4	....
Starch per 0.8 gm. sample by hydrolysis with HCl.	0.0	0.0	0.0	0.0	0.0	0.0
Starch per 0.8 gm. sample by takadiastase method	0.0	0.0	0.0	0.0	0.0	0.0
Percentage reducing sugars.	0.584	0.583	0.348	....	0.311	0.351
Percentage sucrose........	6.05	6.0	8.37	8.44	6.53	6.56
Nitrogen of F_3 as percentage of F_3................	13.9	13.4	12.38	12.31	12.74	12.66
Protein as percentage of dry weight............	33.88	33.04	28.98	28.81	30.02	29.83
Total nitrogen in F_2 as percentage dry weight......	0.185	0.19	0.539	0.536	0.712	0.705
Amino nitrogen in F_2 as percentage dry weight......	0.082	0.083	0.221	0.217	0.672	....
Ammonia nitrogen in F_2 as percentage dry weight...	0.00743	....	0.03773	....	0.04434	....
Amid nitrogen in F_2 as percentage dry weight.....	0.0215	....	0.04376	....	0.05342	....

for a lot of seeds, many of which will swell slowly when the pericarp is removed, and a second lot, part of which may be fully after-ripened but many of which may be still quite retarded.

So early in germination a difference in the lipoid content of the seed has not become apparent, but at later stages, when the hypocotyl is about 1 cm. long, the fat begins to decrease around the hypocotyl and then around the rest of the embryo. A difference in lipoid dispersion as reported by PACK (35) was not established, nor was there any apparent difference in phosphatide content.

The moisture content apparently changes little for intact seeds in contact with a moist seed bed, until after-ripening is practically complete. For air-dry seeds at all temperatures the moisture content reached about 38 per cent after three weeks, rose to 40–42 per cent where it remained until the close of a period of over 100 days, after which it reached from 48 per cent for barely split seeds to 60 per cent for those with elongating hypocotyls.

No difference in acidity was detected by colorimetric methods or by the quinhydrone potentiometer method. In the latter case, since it was necessary to add some redistilled water to the tissue of air-dry seeds, the same amount was added to the imbibed ones. However, the tests were preliminary, and the effect of change in concentration of the solutions was not determined. The pH was apparently between 6.0 and 6.4 throughout the endosperm and embryo until the hypocotyl was nearly 1 cm. long, at which time the outer layer of embryo cells and the sieve tubes showed a pH of 4.4–4.6.

RESPIRATION.—Due to a lack of thermostatic control, the probable error in O_2 data as calculated from the manometer readings seemed too great to warrant the use of these data in determining the respiration quotient. There was very noticeably greater O_2 absorption at 20–22° C. than at the lower temperatures, however, and somewhat greater at 10–14° than at 2.5–7° C.

Tables XIII and XIV show the CO_2 produced in two sets of experiments. In both cases there is a large decrease in CO_2 output at the lower temperatures during the period of after-ripening, with the lowest amount produced at the lowest temperature, that is, that already found the most favorable for after-ripening. The seeds at 2.5–7° C. showed a large increase in CO_2 output, accompanied by rather vigorous germination when placed at room temperature following long cold storage, while those at 10–14° showed some in-

crease in CO_2 but only a feeble tendency to germinate. Several of the woody coverings cracked but germination did not proceed further.

TABLE XIII

MG. CO_2 PER 15 SEEDS PER HOUR (PRELIMINARY)

PERIOD IN DAYS	6° C.	12.5° C.	22° C.
1 to 6th	0.0411	0.0302	0.0489
6–11	0.0307	0.0308	0.08635
11–16	0.0113	0.0213	0.0639
16–22	0.0328	0.0567	0.136
27–34	....	0.0124	0.06623
34–41	0.013	0.0271	0.0326*
41–49	0.0212	0.0405	0.0585
49–58	0.0166	0.0309	0.0581

* At 10° or below for 48 hours.

TABLE XIV

GM. CO_2 PER KG. PER HOUR

PERIOD IN DAYS	2.5°–7° C.		10°–14° C.		20°–22° C.	
	a	b	a	b	a	b
1 to 7th	0.1897	0.26112	....	0.2679	0.395	0.3889
7–14	0.1664	0.154	0.167	0.128	0.243	0.318
14–21	0.0842	0.0912	0.145	0.150	0.174	0.234
21–28	0.0996	0.101	....	0.183	0.558	0.442
28–42	0.0734	0.0577	0.125	0.0799	0.329	0.209
42–56	0.0553	....	0.0866	0.0852	0.178	0.292
56–70	0.0796	0.1049	0.109	0.0595	0.246	0.340
70–84	0.0672	0.0824	0.121	0.03004	0.247	0.244
84–98	0.0924	0.0752	0.0574	0.0652	0.215	0.214
98–112	0.1043	0.1042	....	0.09	0.154	0.370
After 11 days at room temperature (18°–26°C.)	......*	0.237†	0.140	0.215	0.111	......*

* The strain on the apparatus of the fluctuating room temperature produced leaks, so that NaOH overflowed on the seeds, killing a large percentage.

† Fifty per cent of the seeds were well germinated at the end of the period at room temperature.

II. Sambucus canadensis

Two lots of seeds of *Sambucus canadensis*, referred to in the following pages as lot 1 and lot 2, were used for the present study. The first of these was collected in Pennsylvania about October 15. The berries were small and black, of the usual type, the seeds weighing on an average 1.5 mg. each. The second lot was collected in northern Indiana on a low black soil. They ripen about the last of October in this locality, and have a berry somewhat larger than

those from Pennsylvania. The seeds weigh on the average 2.84 mg. each, and look twice as large as those of lot 1.

The seeds were washed free from the pulp, sterilized one-half hour in 0.25 per cent uspulun, and washed thoroughly with distilled water, after which they were put under germinative conditions. About 18 per cent of the seeds in each lot was empty; these floated and were poured off during the washing. Part of each lot was stored air-dry and later used for germinative tests.

GERMINATION RESULTS.—Seeds treated with concentrated H_2SO_4, N/400 acetic acid, and 2, 1, and 0.2 per cent thiourea, urea nitrate, saccharin, and caffeine showed no increase in germination. As may be seen in table XV, those seeds which had been stored at low temperatures, but were not yet after-ripened or fully recovered from dormancy, seemed to benefit from treatment with KNO_3. A solution of N/200 salt was used to moisten the filter paper seed bed in petri dishes. When used with sand it produced no increase over the checks. When added to a non-germinating lot of seeds, those which had been stored air-dry or had ceased germination at an unfavorable temperature, the KNO_3 solution failed to bring about germination.

Seeds planted in the greenhouse during the autumn while sunny days were frequent, and again in the spring, gave good germination, extending from the fourteenth to the thirtieth day after planting. One thousand air-dry seeds of lot 2 put in the 17° C. greenhouse in November remained dormant 56 days, were put at 5–7° for 35 days, then again at 17° in late March as the sun became warm during the day. After 55 days 58 per cent had germinated, at which time the greenhouse temperature had become nearly constant above 20° C. Five hundred seeds air-dried for 13 days gave 55 per cent germination on the hot plate of a 10° oven where the fluctuation was between 10 and 20° C.

Undried seeds of lot 1 without cold treatment gave no germination at 10° or any constant temperature above, but seeds of lot 2 gave scattered germination at all temperatures tried; not exceeding 4 or 5 per cent, however, for any constant temperature. Seeds of lot 1 stored at 10° which showed no germination after 101 days, when shifted to 5°, were germinating well by the 138th day at 5° C.

TABLE XV

GERMINATION UNDER DIFFERENT CONDITIONS OF LOT 2 SEEDS OF SAMBUCUS CANADENSIS STORED AT 0° C. IN MOIST BUILDING SAND

Medium for germination	Temperature (C.)	After 85 days' storage	After 99 days' storage		After 132 days' storage
		6 weeks	2 weeks	6 weeks	20 days
Petri dish H_2O	10	....	23	26	....
Petri dish KNO_3	10	....	30	37	....
Petri dish H_2O	15	17	35	35	73.0
Petri dish KNO_3	15	44	44	57	....
Sand bottle H_2O	15	72	....	....	....
Petri dish H_2O	20	11	36	41	....
Petri dish KNO_3	20	55	52	67	71.0
Sand bottle H_2O	20	71	....	....	....
Petri dish H_2O	24	....	27	28	....
Petri dish KNO_3	24	....	47	49	....
Petri dish H_2O	27	....	18	20	64.0
Petri dish KNO_3	27	....	36	41	....
Petri dish H_2O	32	....	12	13	....
Petri dish KNO_3	32	....	10	17	....
Petri dish H_2O	10–20	....	42	68	....
Petri dish KNO_3	10–20	....	58	81	....
Petri dish H_2O	10–24	....	49	69	....
Petri dish H_2O	10–27	53	46	68	85.0
Petri dish KNO_3	10–27	88	56	82	....
Sand bottle H_2O	10–27	91	....	....	....
Petri dish H_2O	10–32	....	47	62	85.5
Petri dish H_2O	15–20	....	37	54	....
Petri dish KNO_3	15–20	....	57	76	....
Petri dish H_2O	15–24	....	45	57	....
Petri dish H_2O	15–27	42	47	57	73.0
Petri dish KNO_3	15–27	77	60	79	....
Sand bottle H_2O	15–27	90	....	....	....
Petri dish H_2O	15–32	....	50	58	77.0
Petri dish H_2O	20–27	....	33	41	....
Petri dish H_2O	20–32	....	37	45	....
Petri dish H_2O	17 (Greenhouse)	29	47	57	70.0
Petri dish KNO_3	17 (Greenhouse)	87	61	83	....
Sand bottle H_2O	....	87	....	....	....

Tables XVI and XVIII show the percentage of germination for seeds in bottles buried over winter, and for those planted in beds

TABLE XVI

GERMINATION OF LOT 1 SEEDS OF SAMBUCUS CANADENSIS IN SAND BOTTLES AT VARIOUS TEMPERATURES

No. of seeds	Condition of storage	First germination			Days* to completion	Total percentage
		° C.	Days to	Percentage		
1000	5° in moist sand 82 days	10	8	0.3	57	2.8
		15	8	3.5	18	8.0
		20	8	16.2	29	33.3
		24	8	11.4	36	14.1
		27	8	0.0	15	0.3
		32	8	0.0	....	0.0
2500	5° in moist sand 82 days	10–15	....	....	....	0.0
		10–20	15	3.5	64	30.4
		10–24	8	0.8	57	9.0
		10–27	8	14.4	57	87.3
		10–32	8	1.3	57	49.8
2500	5° in moist sand 82 days	15–20	8	4.8	Bad freeze on 18th day	22.0
		15–24	8	....		22.0
		15–27	8	....		53.4
		15–32	8	....		29.1
1000	5° in moist sand 82 days	20–27	8	6.1	29	5.0
		20–32	8	....	29	12.0
2500	5° in moist sand 82 days	16–24	....	....	72	50.8

* Total days left at given temperature, 72.

TABLE XVII

GERMINATION OF LOT 1 SEEDS OF SAMBUCUS CANADENSIS BURIED IN SAND BOTTLES IN SAND PIT OCTOBER 18 AND LEFT UNTIL APRIL 15

No. of seeds	Depth in inches	Percentage H_2O in sand April 15	Percentage germination		Days after removal
			While buried	Total	
3700.........	2–4	2.0	57.9	64.1	44
4750.........	$4\frac{1}{2}$–$6\frac{1}{2}$	2.5	18.8	30.0	44
5400.........	$10\frac{1}{2}$–12	3.0	1.1	43.3	44

November 28. The cold winter temperature prepared the seeds for germination, and the fluctuating temperatures of March and April

produced the highest percentage of seed growth. Seeds buried more deeply or those protected by a mulch did not get the benefit of this alternation of temperatures.

All the seeds from the sand pit were placed in the greenhouse, where there was alternation of temperature for about a month. As may be noted, rather rapid germination occurred in all the bottles, but the ones buried more deeply contained the largest number of seeds which were ready for germination as soon as optimum conditions were provided. These optimum conditions, as determined from the experiments shown in table XVI, from moisture determinations

TABLE XVIII

GERMINATION OF LOT 1 SEEDS PLANTED IN COLD FRAMES NOVEMBER 28

No. of seeds	Condition	Inches of mulch	Percentage of seedlings		Mulch removed
			May 5	May 27	
200	Undried	0.0	54.0	59.0	April 1 and 12
		3.0	22.5	22.5	
		6.0	28.5	28.5	
1000	Air-dry	0.0	47.9	47.9	April 1 and 12
		3.0	11.9	22.2	
		6.0	43.0	43.0	

on the sand of different lots, and from catalase studies, seem to be a daily alternation of temperature between 10° and 27°, and a moisture content in the sand of 2.5 to 3 per cent. Results obtained at 10–32°, 15–27°, and 15–32° may be nearly equal to those obtained at 10–27° C. All alternations used consisted of 17 hours at the lower temperature and 7 hours at the higher.

After 130 to 140 days storage at low temperatures, a high percentage of the seeds usually were found split open. These begin growth readily at 20° and 24°, but may be retarded by 27° C. The seedlings are not very susceptible to injury of any kind, and grow quite rapidly at temperatures of 20° or above. The cotyledons expand and serve as leaves until overshadowed by other leaves. The fourth pair of true leaves is usually compound, and did not appear until the eightieth or ninetieth day on seedlings grown at 17–18°.

Seeds of either lot 1 or lot 2 show recovery from exposure to 32° under germinative conditions. From seeds so stored 112 days, 81 per cent germination was obtained within a week after 130 days at 5° (46 per cent had germinated at the close of the 130 days at 5° C.; the remaining 35 per cent after 7 days at 10°–24°).

Alternation alone has never brought air-dry seeds which were put under such germinative conditions to a high percentage of germination, but has caused increased scattered germination in lot 2 seeds. Only following cold treatment of 82–100 days for seeds of lot 2, and 90–130 days for seeds of lot 1 does it prove entirely effective. Catalase studies have shown that alternation has an effect on the activity of air-dried seeds.

Lot 1 seeds were not dormant when washed free from the pulp, for they responded to temperature alternation. The dormancy of seeds of lot 2 may have been due to their having been overheated in the pulp, while enroute by mail. When both lots were air-dry, the seeds of lot 2 responded more quickly to cold treatment or alternation of temperature than those of lot 1.

Most of the seeds subjected to repeated freezing and thawing were killed by the second freeze and thaw, and the killing was complete after the fourth alternation. A few hours' freeze produced a large number of dead seeds among unsplit seeds stored at 15°, although they were allowed to thaw undisturbed at a temperature just above freezing. A brief period of freezing among those ready to germinate and in some cases already split killed the entire lot.

Catalase.—For catalase studies the seeds in most cases were not weighed, but counted in lots of 50 for lot 1 and 25 for lot 2. Where the catalase content was greatly increased, as in the germinated seeds, smaller numbers were used. The seeds were ground for 2 minutes with 2 cc. distilled water, clean quartz sand, and a little $CaCO_3$; diluted with 13 cc. water; and run for catalase in the same way as the *Cornus* seeds. The results appear in table XIX. There is a definite drop in catalase, even below that of the air-dry seed when the seeds of *Sambucus canadensis* are exposed in a moist condition to a temperature of 27° C. Air-dry seeds when placed moist at 0° or 5° steadily increase in catalase content up to the eighty-fifth day, when germination begins. Seeds tested at that time may

show a higher catalase content than ungerminated ones selected at a later date. This is especially true if seeds removed to alternating temperatures are tested for catalase content on removal from the

TABLE XIX

CATALASE ACTIVITY OF SEEDS LOT 1 AND LOT 2 OF SAMBUCUS CANADENSIS STORED UNDER DIFFERENT CONDITIONS

Lot	No. of seeds	Conditions of storage	Cc. O_2 liberated in 10 minutes 1	2
1	50	Air-dry; moist 98 days at 27°	3.0	3.9
		Air-dry 178 days	5.1	5.4
		Air-dry; moist 31 days at 5°	8.4	8.0
		Air-dry; moist 67 days at 5°	8.0	9.4
		Air-dry; moist 178 days at 5°	16.9	17.7
		Moist 104 days at 0°	15.0	9.5
		Moist 104 days at 10°	9.25	....
		Moist 104 days at 20°	7.5	8.0
		Air-dry 104 days	5.0	7.5
		Moist 113 days at 5°	13.2	11.0
		Moist 113 days at 5° and		
		15° for 16 days; 2 mm. radicles	77.0	....
		10–27° C. 16 days; 5–3 mm. radicles	93.0	....
		Moist 113 days; 15° 16 days ungerminated	11.0	11.2
		Moist 113 days; 20° 16 days ungerminated	8.4	10.8
		Moist 113 days; 10–27° 16 days ungerminated	13.2	....
		Moist 113 days; 10–32° 16 days ungerminated	10.8	....
		Air-dry 81 days; 15° 92 days	6.8	5.2
		Air-dry 81 days; 10–27° 92 days	8.7	8.0
2	25	Air-dry; moist 98 days at 27°	3.7	4.0
		Air-dry 178 days	7.3	5.2
		Air-dry; moist 31 days at 5°	8.7	7.6
		Air-dry; moist 67 days at 5°	15.2	15.1
		Air-dry; moist 165 days at 5°	20.0	22.8
		Air-dry 81 days; moist 92 days at 10–27°	14.3	13.4
		Air-dry 86 days; moist 92 days at 10–27°	7.7	8.7
		Moist 113 days at 5° ungerminated	20.4	22.7
		Moist 113 days at 5° germinated	58.5	82.5
		Moist 113 days at 5° 16 days	23.3	....
		Moist 113 days at 5° 20° 16 days	13.9	....
		Moist 113 days at 5° 10–27° 16 days	18.7	18.8
		Air-dry 63 days; 15° 92 days	10.4	9.6

low temperature, and again after the period of alternation has been sufficient to allow germination of most of the fully after-ripened seeds.

Constant temperatures of 15° and above under germinative conditions are less favorable for an increase in catalase than an alterna-

tion of 10° or 15°, but a constant temperature of 15° or 20° is more favorable than air-dry storage. Increased catalase activity is apparently closely correlated with increased seed activity leading to germination, and in most cases may therefore be used as an indication of seed condition.

III. Berberis thunbergii

The present study of seeds of *Berberis thunbergii* is a brief continuation of work done by MORINAGA. Slightly different methods and temperatures were used, but the results check those he reports rather closely.

TABLE XX

GERMINATION OF BERBERIS THUNBERGII AT ALTERNATING TEMPERATURES

° C.	Percentage germination after 36 days*		Collected April 12, 5°, in pulp 18 days
	Air-dry, 151 days	Moist at 5°, 151 days	
5–20	4	68	....
5–24	18	68	37
5–27	....	....	60
10–20	36	72	45
10–24	86	60	78
10–27	....	80	96
15–20	0	57	2
15–24	40	78	19

* Duplicates of 50 seeds used; mold was the chief interfering factor.

In most cases the seeds were mixed with moist peat in wide mouthed bottles. Those seeds gave better results than seeds mixed with building sand, and germination in quartz sand was obtained only in the bottom of the bottle.

As MORINAGA found, *Berberis thunbergii* seeds germinate best at an alternation between 10° and a higher temperature, but the higher temperatures found equally favorable were 20°, 24°, and 27° C. A period of over 100 days at 5° will give good germination. A brief cold period of 30 to 40 days seems to favor germination at the less favorable temperatures, but has little or no effect at the optimum alternation.

Seeds planted in cold frames over winter (table XXII) tended to

TABLE XXI

GERMINATION OF BERBERIS THUNBERGII UNDER DIFFERENT CONDITIONS

No. of seeds	Date of collection	Condition of storage	Germination: Medium	Germination: ° C.	Total percentage	Days
240 240	November 12	Dry in pulp 120 days at 8°	Peat bottle	5 10 10–25	93.8 61.2 98.6	101 101 38
450 420 450 300 450 450 100	March 10	....	Peat bottle	5	97.1	63
		....		....	90.0	63
		....		10	35.0	63
		....		....	30.0	63
		....		15	......	101
		....		....	......	11
		Moist peat 29 days at 5°	Petri dish	24, then 10–24	16.0 71.0	24 35
		at 10°	Petri dish	24, then 10–24	4.0 70.0	24 35
		at 15°	Petri dish	24, then 20–24	0.0 29.0	24 35
50	November 14	Air-dry, then moist peat April 30 (61 days) 5°	Petri dish	10–24	82.0	17
50	November 28	....	Petri dish	10–24	88.0	17
			Petri dish	10–24	92.0	17
			Petri dish	10–24	99.5	17

TABLE XXII

GERMINATION OF SEEDS OF BERBERIS THUNBERGII PLANTED IN COLD FRAMES NOVEMBER 28

No. of seeds	Condition and date of collection	Inches of mulch	Date of removal	Percentage seedlings: May 9	Percentage seedlings: May 26	° C.*
1000....	Seeds, November 12	0	....	53.2	60.3	......
700....	Berries, November 12	0	....	53.9	81.3	......
1000....	Seeds, November 28	0	....	78.1	....	......
700....	Berries, November 28	0	....	66.2	96.0	......
1000....	Seeds, November 12	3	April 1 and 12	65.3	....	3
700....	Berries, November 12	3		52.6	63.0	3
1000....	Seeds, November 28	3		58.4	....	3
700....	Berries, November 28	3		50.4	57.7	3
1000....	Seeds, November 12	6	April 1 and 12	49.1	....	3–5
700....	Berries, November 12	6		65.6	71.9	3–5
1000....	Seeds, November 28	6		55.0	....	3–5
700....	Berries, November 28	6		85.6	....	3–5

* Atmospheric minimum was −3° F.

germinate best in the unmulched beds, due probably to the influence of fluctuating temperatures, which become effective there sooner than where the mulch must be removed. However, the germination of *Berberis thunbergii* is rather quick, and therefore was fairly complete by the end of April when the temperature became higher. Seeds planted in the berries were retarded a week or two, but the results are not very significant, since the berries might contain either one or two seeds.

Damping off by *Pythium* was checked by Dr. C. R. ORTON, who applied a 0.25 per cent solution of uspulun to the rows of seedlings on May 5 and again on May 12.

Discussion

The seeds studied differ rather widely in type and in their requirements for germination, but have some characteristics in common. All three fruits have a pulpy outer layer which must be eliminated prior to storage; otherwise considerable or even total loss may result from bacterial and other fungal infection.

In every case cold moist storage favors germination more than dry storage. In *Berberis* the chief effect is probably due to retention of water to such a degree that the time required for germination does not need to include a period of preliminary imbibition.

Cornus seeds, during the long period of 90 to 130 days of cold moist storage, exhibit interesting changes in capacity to germinate, catalase activity, respiration, and chemical composition. Increased capacity for germination is accompanied by increased catalase activity, and an increase in starch, sucrose, and soluble protein, but by decreased respiration continuing until germination actually begins. At higher temperatures (above 15°) on the other hand, no increase in readily usable food materials was noted, catalase activity dropped rapidly to a point much below that for air-dry seeds, respiration remained fairly high, and the seeds did not after-ripen. HARRINGTON (18) notes an increase in respiration with increased dormancy at higher temperatures for the apple seed, and a decrease at lower temperatures. He suggested that there is probably an increase in acids and sugars at the lower temperatures.

One would conclude from these facts that after-ripening is rather closely tied up with respiration. At the higher temperatures

the readily oxidized food materials may be used in respiration as fast as they are formed, while at the lower temperatures, necessary for after-ripening, the reduced respiratory activity allows them to accumulate in sufficient quantities to initiate growth.

Any relation between catalase activity and respiration is more difficult to demonstrate. The catalase is evidently used up at higher temperatures, where the respiration rate remains high. However, exhaustion of the catalase supply in the presence of aerobic respiration products does not stimulate production of the enzyme, until catalase activity again keeps pace with respiration, as Mrs. RHINE (37) has suggested in an application of LOEW'S theory to her results. At low temperatures the drop in respiration is abrupt, after which the rate remains constant, while the increase in catalase activity is as gradual and steady as the decrease at higher temperatures, suggesting an accumulation of the enzyme. As germination begins at the close of after-ripening, both respiration and catalase activity show marked acceleration, but on account of the great individual variation in *Cornus* seeds, any preliminary drop in catalase, as observed by Mrs. RHINE in the early stages of germination, might not be detectable.

SHERMAN (39) reports a similar rise in catalase activity during after-ripening of *Crataegus mollis* seeds, accompanied by a decline in respiration rate after the sixth or seventh day. Although catalase does seem to be closely related to vigor and capacity for high metabolic activity, it does not, at least in some cases, follow the respiratory curve or even seem to be related to it, either during after-ripening or during the storage of moist unafter-ripened seeds at unfavorable temperatures.

COLE (6), citing DIXON'S work, suggests that catalase by destruction of H_2O_2 formed during oxidation conserves other oxidizing enzymes. Thus, he suggests, the almost universal distribution of catalase may be related to the conservation of oxidizing enzymes.

KINZEL (25), as the result of extensive investigations with seeds of numerous families, has arrived at the conclusion that the habitat of the parent plant is closely correlated with the germination behavior of the seeds. In connection with this hypothesis he has especially emphasized two factors, cold and light. The seeds of plants

from alpine habitats are especially prone, he believes, to require before germination one or more seasons of freezing through, because for generations such seeds have been exposed to the low winter temperatures of the alpine meadows. On the other hand, seeds of the same genera but of lowland species may require no cold season, or at most only a short one to prepare them for germination. One of the genera he particularly observed during his earlier studies (24) was *Sambucus*. He regards this as a "Frost-keimer," requiring two seasons of freezing for germination. The results reported in the body of this paper do not agree with Kinzel's conclusions. *Sambucus* seeds not only do not require freezing, but in some cases do not require even a short cold treatment at a temperature above freezing. The most important factor influencing their germination, unless they are in a state of imposed dormancy, seems to be a suitable alternation.

Furthermore, "Durchfrieren," which is taken to mean actual freezing of the seed, is not only unnecessary but even injurious. Dry seeds are apparently uninjured but are not after-ripened at freezing temperatures. Moisture is essential to after-ripening. Whenever imbibed seeds of the species studied are exposed to temperatures below freezing long enough to freeze through, killing results. Undercooling may occur in some cases, or the moisture content of the seed may be so low that total killing does not result when the sand or soil about the seed is frozen. Since the power of the seed colloids to hold water is tremendous, ice does not form in the seed unless a relatively high moisture content has been attained. However, after-ripening in seeds of *Cornus florida* is retarded as the temperature drops to 0° C. Even when ice formation does not occur, the lower temperature and lower moisture content are less favorable for after-ripening than a temperature nearly 5° above freezing accompanied by sufficient moisture.

Pack (33) reports similar results for *Juniperus* seeds. Although exposure to $-5°$ C. was not sufficient to injure the ungerminated seeds, no forcing action was noticed. Seeds which were germinated were killed by a rather brief exposure to freezing.

As may be concluded from the data presented, and from the results reported by other workers, freezing is not necessary to break

the hard coats of stony seeds. In most cases the swelling seed exerts a pressure sufficient to burst the covering. In other cases the effects of alternate wetting and drying or weathering weakens the coat so that it may easily be broken. TUKEY (41) found that freezing soaked peach pits did not crack the coats, even when prolonged sufficiently to kill the seed.

Summary

1. Seeds of *Cornus florida* are dormant when the fruit is mature. This dormancy, which is caused by embryo and endosperm characters, cannot be broken by treatments with acids, ether, or ethylene.

2. A period of 100–130 days at 0°, 5°, or 10° C. is most effective for after-ripening, which may be defined as that change in the endosperm and embryo of the seed in consequence of which the seed is able to germinate at ordinary temperatures (15°–27°).

3. The changes apparent during after-ripening are increase in starch, sugar, and amino acids, with little or no change in fats, acidity, or phosphatides.

4. Germination consists of a rather sudden swelling of the embryo and endosperm, sufficient to break the stony coat followed by the elongation of the hypocotyl. The cotyledons remain within the endosperm, acting as haustoria (43) until all the food reserves are exhausted, after which they function as leaves.

5. After-ripening conditions of cold moist storage may be obtained in mixtures of seeds with sand or peat placed in refrigerators, by burying the containers, or by planting the seeds in beds mulched during the winter.

6. Under natural conditions germination occurs in the spring among those seeds which have lain throughout the winter in moist sheltered spots well covered with leaves.

7. Catalase activity parallels after-ripening very closely. It may be considered an indication of the direction of change, whether toward deeper dormancy or toward germination.

8. Catalase activity has also been used to detect dead seeds.

9. Respiration falls early in the period of exposure to low temperatures for after-ripening, and remains low until germination begins. At higher temperatures (above 15°) it remained constant and fairly high for the length of time tried (124 days).

10. Seeds of *Sambucus canadensis* may or may not be dormant when collected. If not dormant they will grow in 14–30 days at a daily temperature alternation of 10° for 17 hours and 27° for 7 hours or at a similar range of alternation. If dormant they require a period of 85–100 days at 0° or 5°, after which they will germinate on subjection to the necessary alternation, or at 0°–5° if left long enough.

11. Such seeds when once split grow very well at higher constant temperatures.

12. Exposure to higher temperatures under germinative conditions deepens the dormancy in seeds of both *Cornus florida* and *Sambucus canadensis*, as indicated by their failure to germinate and their drop in catalase content. Recovery may be brought about in each case by a period of 130–140 days at 5° C.

13. Seeds of *Berberis thunbergii* resemble those of *Sambucus* in that they require alternating temperatures for germination. They differ in that they require only a very short cold treatment or none at all for germination.

14. Seeds of either *Sambucus* or *Berberis thunbergii* planted out-of-doors in the autumn, protected from freezing, and exposed to the fluctuating temperatures of early spring, reach from 60 to 70 per cent germination.

15. Freezing in no case favors after-ripening, and always kills large numbers of imbibed seeds.

16. Storage in the pulp, especially in a moist condition, leads in all seeds studied to bacterial and other fungal infection, resulting in a high percentage of killing. This is particularly true for *Cornus florida*.

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DISSOLVED GASES IN WATERS OF SOME PUGET SOUND BOGS

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(WITH TWO FIGURES)

Introduction

This paper reports data on the composition of dissolved gases occurring in fifteen different samples of bog water collected from four sphagnum bogs near Seattle, Washington, and the constituents of two samples of gas collected directly from one of these bogs. The discussion deals with the probable relation of the dissolved gases to the growth of plants in the bogs. Since the four bogs differ somewhat in their physical character and in their flora, a description of each bog is given.

Esperance Bog is situated near the southern boundary of Snohomish County, about four miles north of the city limits of Seattle, and nearly half a mile west of the new Pacific Highway. It has an area approximating five acres, including a small pond near the center. The bog is surrounded by low hills of glacial till, which, prior to logging operations of some years ago, were covered with a coniferous forest. Much of the surface of the bog is composed of living *Sphagnum*, and under this is several feet of dead *Sphagnum* only slightly disintegrated. The deeper layers consist of a very wet brown peat containing remains of marsh plants.

The plants constituting the present bog flora are mainly *Ledum groenlandicum*, *Kalmia polifolia*, *Oxycoccus oxycoccus*, *Drosera rotundifolia*, *Carex* sp., and *Eriophorum russeolum*. On most of the bog these plants are in the living *Sphagnum*, although in a few places the *Sphagnum* at the surface is dead. The forest growth consists mostly of *Tsuga heterophylla* and *Pinus monticola*. The former species is much more abundant and the trees are small, many of them being not more than 6 feet tall, while the latter are rather scarce, but some of its specimens reach a height of 20 feet. Both species show some seedlings. *Nymphaea polysepala* and *Brasenia schreberi* are abundant

on the margin of the pond surrounded by the bog. Bunches of the former are also found in the bog with *Sphagnum* and the other bog plants crowding closely around them. The bog is rapidly encroaching upon the pond, *Kalmia* being a conspicuous pioneer in the advance, often accompanied by *Oxycoccus*, and usually closely followed by *Sphagnum* and *Carex*. The entire bog is very wet, and there are many holes and natural ditches in which water stands at practically the level of the pond. Much of the bog, particularly near the pond, is still in the floating mat stage of succession. These data seem to indicate that the lake or pond formerly occupied a large part of the depression, and that much of the bog has been formed by the advance of the floating mat of vegetation upon the body of water.

Soundings to a depth of 25 feet, made with a Davis peat sampler, did not reach solid soil. At from 23 to 25 feet samples of well disintegrated brown peat were obtained. These contained fibers of marsh plants, apparently *Carex*, and some whitish material which contained diatoms. At depths from 3 to 23 feet the material is very soft, in many places having the consistency of a thick soup.

Sunnydale Bog is situated about four miles south of the city limits of Seattle, just east of the Des Moines Highway. Low hills of glacial till surround it on three sides, while on the south there is an elevation of just sufficient height to prevent drainage. The coniferous forest that covered the hills has been logged off. The bog has an area of 20 or more acres, and surrounds a small lake or pond situated near the east side, having an area of about one acre. The main portion of this bog has grown up to an elevation of a foot or two above the pond, and is much drier than Esperance Bog. The surface is largely composed of *Sphagnum*, but this is mostly dead, excepting near the pond and in a few places near the marginal ditch.

The bog is completely encircled by a natural marginal ditch of the usual type found around mature sphagnum bogs in the Puget Sound region. This ditch is over 150 feet wide in places, and is very wet even in midsummer. Water in the ditch has a depth of several feet in midwinter. The vegetation of the ditch consists in some places of an almost pure stand of *Spiraea douglasii*, in other places of a dense growth of *Carex* with some *Menyanthes trifoliata*, while in others, where there is less water, it consists largely of *Thuja plicata* and

Tsuga heterophylla with some *Pseudotsuga taxifolia*. In many places throughout the ditch there is considerable *Alnus oregona*, *Salix* sp., and *Pyrus rivularia*.

On the margin of the pond there is a dense growth of *Polygonum* sp., *Comarum palustre*, and *Nymphaea polysepala*. In some places the water under the leaves of *Nymphaea* is filled with a dense growth of *Riccia fluitans*, and in other places there is a good deal of *Utricularia vulgaris* and *Myriophyllum* sp. The pond is 27 feet in depth in the center and 18 feet near the eastern margin.

All of the bog plants found in Esperance Bog are also found in the Sunnydale Bog. In addition, *Dulichium arundinaceum* and *Juncus ensifolius* are common in the wetter portions near the pond, and *Typha latifolia* is found in a few places. *Nymphaea polysepala* is common in this bog, as in Esperance. In the younger portions of the bog near the pond, it grows vigorously in openings several feet in diameter, but the bunches are small and are almost crowded out by the bog association in the mature stages of the bog farther back from the pond.

Much of this bog has reached a rather mature stage, and has a coniferous forest succession of *Tsuga heterophylla* reaching a height of 25 feet, together with some *Thuja plicata* which is not as tall. Seedlings of both of these species are common. There are also occasional small trees of *Pseudotsuga taxifolia*.

Over fifty soundings have been made in this bog, and numerous samples have been taken. In many places west of the pond bottom was not reached at 31 feet, and a layer of whitish material containing diatoms was found at 27 feet. East of the pond, at hole no. 1, where analyses were made of the dissolved gases in the water, the bog is 31 feet deep and rests on blue clay mixed with coarse sand. In the northern portion of the bog, at hole no. 2, where other analyses for the dissolved gases were made, bottom was not reached at 31 feet. Gravel was encountered at 13–17 feet at several points southeast of the pond. The surface as well as the deeper layers of this bog is very similar to that of Esperance.

White Center Bog has an area of less than 10 acres, and is situated about a mile west of White Center, just south of the city limits of Seattle. The bog lies in a north and south valley, and hills of gla-

cial till rise rather abruptly from it on the east and west. North and south of the bog the land is flat, and has an elevation just sufficient to prevent drainage. The coniferous forest that once surrounded the bog has mostly been removed, while a road has been graded along the southern side, covering a part of the marginal ditch. There is no pond in this bog. The surface is fairly dry in summer, but water stands on portions of it in winter.

The flora of this bog differs somewhat from that of the other three bogs described. There is very little *Sphagnum* on the surface, either living or dead, and no *Kalmia* or *Drosera* has been found. *Ledum* and *Oxycoccus* are abundant, and in places there is a dense growth of *Spiraea* and considerable *Carex*. Young trees of *Tsuga heterophylla* up to 8 or 10 feet in height are numerous, and *Thuja plicata*, mostly of a shorter height, are common. A few small specimens of *Pseudotsuga taxifolia* are also found. The usual marginal ditch surrounds the bog.

Three soundings were made in different parts of the bog, and blue clay was found at depths of 14 feet in all cases. A hole 4 feet in depth was dug in the bog in June, when the water table was low enough to permit such digging without encountering much water. The upper 6 inches was found to consist of fibrous peat with no *Sphagnum*. The next 12 inches was pure *Sphagnum* only slightly disintegrated. The peat below that was uniform in character. It was dark brown, and the remains of a *Carex*-like plant were abundant in it.

Ronald Bog has an area of more than 20 acres, and is situated about 3 miles north of the city limits of Seattle and one-half mile east of Ronald station. It is surrounded by low hills of glacial till and there is no drainage from it. The coniferous forest that covered the hills has been cleared and several ranches now occupy portions of the land adjacent to the bog. An automobile road crosses the northern end of the bog on a fill, and portions of the marginal ditch have been used as a dumping ground for refuse. On the eastern boundary the marginal ditch widens into a rather extensive marsh, characterized mostly by dense growth of *Spiraea douglasii*, with a quantity of *Populus tremuloides* reaching a height of 15 feet.

The bog has the usual flora of *Ledum groenlandicum*, *Kalmia*

polifolia, *Oxycoccus oxycoccus*, and *Drosera rotundifolia*. There is still some living *Sphagnum*, although this has grown less and less as the bog has dried out through its modification by human agencies during the last 15 years, and much of the surface now consists of dead *Sphagnum*.

The forest growth in this bog consists mostly of *Pinus monticola*. Trees of this species are numerous, and commonly reach a height of 30–35 feet in the older portions of the bog. There is also considerable *Tsuga heterophylla*. Seedlings of both of these species are common. A few small specimens of *Pseudotsuga taxifolia* are found. Good trees of *Picea sitchensis* were common in the marginal ditch prior to clearing, but none were found in the bog itself.

The upper layer of the bog to a depth of 2 feet or more consists of *Sphagnum* in a good state of preservation and brown in color. Below this is brown peat free from *Sphagnum*, but containing remains of a *Carex*-like plant and some fragments of woody plants. Eight soundings were made on a north and south line in the central portion of the bog, but not extending to the marginal ditch. Sand was reached at 7 feet in the most northerly sounding, and at gradually increasing depths up to 17 feet for the sixth sounding. Blue clay was reached in the seventh sounding at a depth of 26 feet, and in the eighth at 27 feet. The peat is less watery than that of Esperance Bog and Sunnydale Bog, but slightly more watery than in White Center Bog.

Collection of samples

Holes about 2 feet in diameter were dug in the bog, and collections were made from the water that seeped into them from the substratum. In Esperance Bog these holes were about 2.5 feet deep and they were filled with water in 15 minutes. In other bogs deeper holes were dug, 4 feet in Ronald Bog being the deepest, and water accumulated in them much slower, a wait of an hour being necessary in many cases.

In securing samples of bog water, a 2.5 liter glass-stoppered bottle was used, loose pieces of *Sphagnum* and other plant remains being prevented from entering the bottle by means of wire gauze. The stopper was replaced in the bottle while it was submerged, and on being brought to the surface it was thoroughly sealed. The bottle was then

TABLE I

QUANTITIES OF VARIOUS GASES DISSOLVED IN BOG WATERS

Description of sample	Date collected	Gas dissolved per liter of water ml. corrected to 760 mm. 0°C.	Carbon dioxide			Oxygen			Methane			Nitrogen		
			Per cent by volume	p.p.m.	ml. per liter	Per cent by volume	p.p.m.	ml. per liter	Per cent by volume	p.p.m.	ml. per liter	Per cent by volume	p.p.m.	ml. per liter
Esperance Bog														
Hole 1	Oct. 31, 1925	64.4	60.4	76.9	39.2	0.0	0.0	......	22.3	10.3	14.4	17.3	13.9	11.1
Hole 2	Nov. 25, 1925	58.5	62.2	71.9	36.7	0.3	0.3	0.2	9.4	3.9	5.5	28.1	20.6	16.5
Hole 3	Feb. 27, 1926	60.6	57.0	68.3	34.8	0.0	0.0	......	21.5	9.3	13.1	21.5	16.3	13.1
Sunnydale Bog														
Hole 1, sample 1	Feb. 27, 1926	59.9	60.4	71.5	36.5	1.1	0.9	0.6	11.6	5.0	7.0	27.0	20.2	16.2
sample 2	Feb. 27, 1926	59.5	61.0	71.7	36.6	1.1	0.9	0.6	9.7	4.1	5.7	28.2	21.0	16.8
Hole 2, sample 1	Feb. 27, 1926	45.4	44.2	39.7	20.3	1.9	1.2	0.8	17.3	5.6	7.9	36.6	20.8	16.6
sample 2	Feb. 27, 1926	46.5	43.9	40.4	20.6	2.0	1.3	0.9	17.6	5.9	8.3	36.5	21.2	17.0
White Center Bog														
Hole 1, sample 1	Nov. 25, 1925	38.9	46.5	35.8	18.3	2.7	1.5	1.1	0.0	0.0	......	50.8	24.7	19.8
sample 2	Nov. 25, 1925	39.2	47.3	36.7	18.7	3.2	1.8	1.3	0.0	0.0	......	49.5	24.3	19.7
Hole 2, sample 1	Feb. 27, 1926	35.2	46.6	32.4	16.5	0.0	0.0	......	0.0	0.0	......	53.4	23.5	18.8
sample 2	Feb. 27, 1926	35.1	46.6	32.3	16.5	0.0	0.0	......	0.0	0.0	......	53.4	23.5	18.8
Ronald Bog														
Hole 1, sample 1	Feb. 27, 1926	35.1	47.7	33.1	16.9	0.2	0.1	0.1	0.0	0.0	......	52.1	22.9	18.3
sample 2	Feb. 27, 1926	37.1	47.1	34.5	17.6	0.0	0.0	0.0	0.0	0.0	......	52.9	24.5	19.7
Hole 2, sample 1	Feb. 27, 1926	48.5	58.2	55.8	28.5	1.1	0.8	0.6	0.0	0.0	......	40.7	24.7	19.8
sample 2	Feb. 27, 1926	48.9	57.6	55.7	28.5	0.9	0.6	0.4	0.0	0.0	......	41.5	25.4	20.3

taken immediately to the laboratory and stored in a refrigerator until preparations were complete for analysis.

The gas from Esperance Bog was collected by immersing and inverting the top of a large funnel, 30 cm. in diameter, in the water that had accumulated in the holes. The funnel was connected with a gas sampling bottle by means of rubber tubing and the entire apparatus completely filled with the water. A long pole was then run into the soft peat at the bottom of the hole and worked back and forth until sufficient gas was collected.

Holes no. 1 and no. 3, table I, in Esperance Bog were in a later stage of bog succession. Hole no. 2 in this bog was nearer the pond in a much earlier stage of the succession. Hole no. 1 in Sunnydale Bog

TABLE II

ANALYSES OF GASES COLLECTED FROM ESPERANCE BOG

Sample no.	Percentage					
	Carbon dioxide	Oxygen	Carbon monoxide	Unsaturated hydrocarbons	Methane	Nitrogen
1	3.8	0.0	0.0	0.0	30.6	65.6
2	4.0	0.0	0.0	0.0	37.4	58.6

was west of the pond and in an earlier stage of the succession than hole no. 2, which was located in the northeast portion of the bog. The two holes in Ronald Bog were 100 feet apart, but were practically in the same stage of the succession. In White Center Bog hole no. 1 was near the marginal ditch, while hole no. 2 was near the center of the bog.

The two samples of bog gas, the analyses of which are shown in table II, were collected from the same hole in Esperance Bog (no. 1). Sample no 1 was collected first, and represents the gases accumulated in the upper strata of the bog, while no. 2 was obtained by disturbing the peat at greater depths.

In all cases the temperature of the water at the time of collection of samples in any of the bogs was never above 10° C. or below 4° C.

Apparatus used

After a study of the literature dealing with the analysis of gases dissolved in natural waters, a modification of the method described

by TREADWELL and HALL[1] was finally utilized. Fig. 1 shows the modified type of apparatus employed in this investigation, while a description of its manipulation is outlined below.

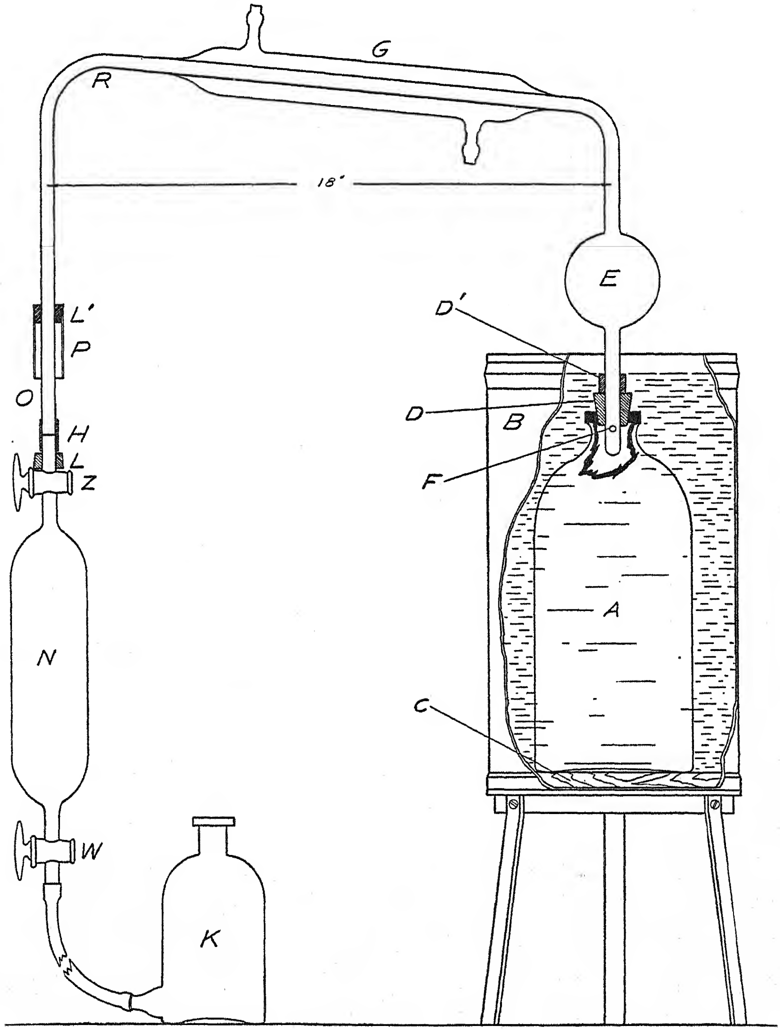

FIG. 1

The bottle *A*, containing about 2.5 liters of the bog water to be analyzed, was placed in the boiler *B*, so that it rested upon the wood-

[1] TREADWELL and HALL, Analytical chemistry. Vol. II, 630–632. 6th ed.

en stand *C*. The employment of the latter was necessary as it insured proper circulation of the heating medium of the boiler. This prevented cracking the container by sudden temperature changes, and eliminated the undesirable effects of violent bumping. The bulb *E*, together with the two pieces of tubing sealed into it, were made of pyrex glass and are partly illustrated in detail in fig. 2. One stem of the bulb, sealed at the end but having a hole of sufficient size at *F*, was adjusted to position no. 1 (fig. 2) and immersed in distilled wa-

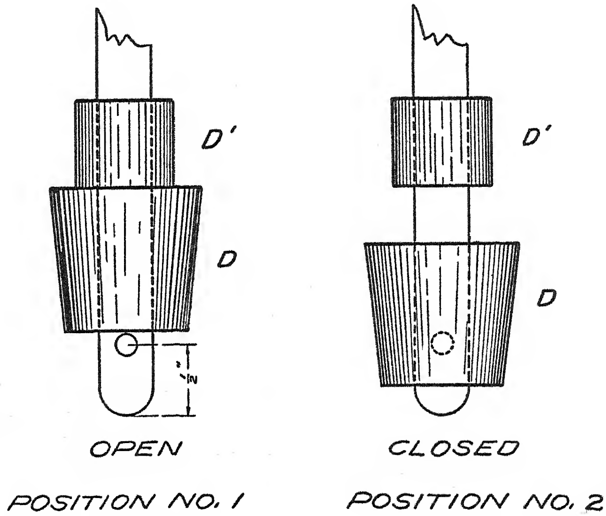

FIG. 2

ter. A rubber stopper (*D*) served to regulate the proper adjustment of the hole (*F*). A suction was applied at the end of the stem *O*, and water drawn into the bulb (*E*) until it was half filled. The hole was then adjusted to position no. 2 (fig. 2). Heat from a Bunsen burner was applied at *E* to thoroughly boil the water in the bulb. During this process the condenser (*G*) was free of any water that might have served as a cooling agent. Boiling was continued for at least five minutes, in order to expel all the air from the tube *O*.

The services of two persons were required to connect the various pieces of apparatus. One held the piece containing the bulb with its pyrex tubing and condenser and kept the water in the bulb boiling vigorously, while the other made the proper connection at *H* with

gas holder *N*, by means of heavy-walled rubber tubing. The latter was carefully wired. The small glass tube *P* was lowered from the rubber stopper L^1 to *L*, and filled with water or mercury, thus giving a water or mercury seal to the connection. The entire process of connecting and sealing was performed in less than a minute. When complete, the source of heat at *E* was withdrawn.

The glass stopper of the container was then removed, and the space occupied by it filled with distilled water. The rubber stopper (*D*) was inserted into the bottle. The boiler was then filled with a saturated sodium chloride solution so as to immerse completely the rubber stopper. This gave a water seal to the connection. The small stem of the bulb was adjusted to position no. 1 (fig. 2), and the connection made with *D* was tightened and secured with wire. The hole *F*, in position no. 1, was arranged so that it was just below the stopper. This was very essential as it eventually permitted the complete removal of the gases from the bottle as they came out of solution.

The temperature of the solution in the boiler was raised and so regulated that the water in the container was kept boiling. The usual device for maintaining a constant level was attached to the boiler.

Water was permitted to circulate through the condenser as soon as the heat was applied to the boiler. At the same time the stopcocks *Z* and *W* were opened, and the mercury, which had completely filled the gas holder *N* and the small tube projecting from it at *H*, flowed into the reservoir (*K*). A partial vacuum was thus produced in the system, and any tendencies for leaks at *H* and *D* could easily be noted and rectified.

The bog water in the container *A* was subjected to boiling temperature for at least an hour and until all dissolved gases had been removed. As the result of heating the 2.5 liters of bog water, an expansion of about 90 ml. was obtained. The bulb (*E*), which had a capacity of 200 ml., readily accommodated the excess water, and also provided ample space into which large bubbles from the container burst without being carried over to the gas holder *N*. The water condensing in the tube contained in the condenser ran back into the bulb.

After the gases had been completely boiled off, the solution in the boiler was cooled sufficiently so as to permit the removal of the stopper. Care was taken that the hole was kept well below the sur-

face of the water in the boiler. The water in the bulb was boiled for a minute to insure the removal of any dissolved gases. All of the gas in the tube (*O*) was then rapidly drawn into the container *N*.

The method, while somewhat tedious, yielded excellent results after sufficient skill had been acquired in the manipulation of the apparatus. Care was exercised in removing all the air from the tube (*O*), the bulb (*E*), and its complete displacement by mercury in all parts of the gas holder *N*. When the connection was made at *H* a towel was wrapped around the gas holder, which protected it from the steam and an occasional drop of hot water. Wiring of the rubber tubing at *H* and of the stopper *D* was found to be important, and the use of the water and mercury seals essential. In transferring the residual gas in the tube *O*, the amount of water entering the gas holder was kept at a minimum by the following manipulation. The water was drawn through the tube until it reached the bend at *R*. Stop cock *Z* was closed, and then the pressure of the gas in the container greatly reduced. The stop cock was then opened again and closed just as soon as all of the gas in the tube was transferred.

Methods of analysis

The total volume of the gas obtained from the samples of water was measured. The necessary corrections for temperature, pressure, and water vapor were made, and the amount of gas, calculated for standard conditions, dissolved in a liter of the water was determined. Portions of the gas were carefully analyzed in the usual manner for carbon dioxide, oxygen, carbon monoxide, unsaturated hydrocarbons, and methane. The residual gas was reported as nitrogen. Hempel burets and pipets were utilized.

Discussion

The outstanding facts shown in tables I and II are: (1) The considerable quantities of methane in the samples of water from each bog containing a lake or pond, and its absence in bogs not containing such open bodies of water. The former type of bog is designated here as a "wet bog," while the term "dry bog" refers to one of the latter type. (2) The relatively large amounts of carbon dioxide found in all of the samples of bog water. (3) The presence of greater quantities of carbon dioxide in the wet bogs than in the dry bogs. (4) The en-

tire absence of oxygen in five of the samples and the presence of only very small amounts in the other ten. (5) The complete absence of carbon monoxide and unsaturated hydrocarbons in all of the waters. (6) The greater quantities of dissolved gases in the waters of wet bogs than in those from dry bogs. (7) The large amount of dissolved nitrogen (residual gas) in samples from dry bogs as compared with that in the wet bogs. (8) The greater concentration of methane in the deeper layers of Esperance Bog as compared with the more superficial layers. (9) The small amount of carbon dioxide and the large amount of methane in the gas samples collected by agitation of the sphagnum peat under the waters in Esperance Bog.

The injury to many land plants by submergence of their roots in water is well known. Plants that ordinarily grow in soil that is merely moist are usually killed by such treatment, although plants that flourish in very wet habitats will stand rather prolonged submergence of their roots. BERGMAN[2] showed this by experiment, and his results agree with the observations of the writers on agricultural plants where submergence by the overflow of streams occurred. This is also in accordance with their observations on native plants growing in low places in the Puget Sound region, where flooding in winter and spring was frequent.

The four bogs discussed in this paper differ considerably in the level of the water table at different seasons. In Esperance Bog the water table is practically at the surface during the entire year. *Carex* flourishes in the bog stage here, but other plants that ordinarily grow in marshes have not established themselves. *Nymphaea*, which flourishes in the pond, remains in the early bog stage but not in the later. *Brasenia* is abundant in the pond but is not found in the bog. In Sunnydale Bog the conditions in regard to the water table close to the pond are similar to those in Esperance Bog, but farther back, where the bog stage is more mature, the water table is low enough in summer so that the roots of plants growing there are not submerged. *Nymphaea* behaves in the same manner in this bog as it does in Esperance Bog. *Dulichium arundinaceum* and *Typha latifolia*, whose roots are well known to withstand considerable submergence, flourish in the earlier stages of this bog, where the water table is high.

[2] Ann. Botany 34:13–33. 1920.

Land plants have only in rare instances established themselves, however, even where the water table is low.

In Ronald Bog the water table is low enough so that the roots of plants are not submerged in spring and summer, yet land plants, with the exception of *Pteridium aquilinum*, have not established themselves, in spite of the fact that the bog has become much drier by the clearing of the surrounding forest. White Center Bog is flooded in winter, but the water table is lowered considerably in summer. It lacks some of the usual bog plants, while *Spiraea douglasii*, a marsh plant, is abundant. It is to be remembered that the surface layer of peat in this bog is not sphagnum peat, although the second layer is composed of this material. Evidently the stage of succession must be considered as a factor in this bog.

However, the growing season in the Puget Sound region is long, and the roots of many plants grow considerably, even in the winter months. Flooding at this time and in the spring may have greater effects upon bog flora of this region than in places where roots are dormant for a longer time. While the occurrence of certain plants in the four bogs and the absence of other plants growing in the immediate vicinity correlate to a certain extent with submergence of roots, they do not entirely do so, and other factors must be considered.

The necessity of an oxygen supply around the roots of plants and the injurious effects of large amounts of carbon dioxide are well established. BERGMAN found that potted plants are injured or killed by replacing the soil atmosphere with carbon dioxide, and that injury to plants by submergence in many cases is obviated by aeration of the water. CANNON[3] carried out experiments on root growth in relation to a deficiency of oxygen and an excess of carbon dioxide in about 30 species, including a number of agricultural plants. The data on carbon dioxide in the substratum of Puget Sound bogs are not directly comparable with CANNON'S results. The analyses shown in table I give the percentage of carbon dioxide found in the gas dissolved in the waters, while he used it as a soil atmosphere. The difficulty of conceiving how carbon dioxide can be so injurious to the growth of the roots of land plants, as is shown in CANNON'S results, and not be likewise injurious to them when dissolved in the

[3] Carnegie Inst. Wash. Publ. 368. 1925.

soil water, is removed by a consideration of the following equilibrium conditions between the carbon dioxide and the soil water: $\underset{\text{(gas)}}{CO_2} \rightleftarrows \underset{\text{(dissolved gas)}}{CO_2 + H_2O} \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^-$. While a portion of the carbon dioxide is in solution, it is evident that a relatively large portion has reacted with the water, forming an entirely different substance. This is particularly true of soil solutions where a degree of saturation is by no means approached. The formation of the acid gives rise to the factor of acidity, which may have a decided effect upon the growth of roots. Furthermore, an increase in carbon dioxide reduces the partial pressure of oxygen, and thus decreases the solubility of the latter gas.

BERGMAN has determined the amount of carbon dioxide in the waters of a lake, a swamp, and a bog in Minnesota. Water was taken from under the *Carex-Calamagrostis* "associes" and the *Larix-Picea* "associes" in the swamp bordering a lake. *Sphagnum* and other mosses were abundant in both "associes," and under *Carex* and also under *Andromeda* and *Sphagnum* in the bog. The amount found by BERGMAN for the Minnesota bog varies from 11.4 to 20.1 p.p.m., average of all results being 15.1 p.p.m., while the amount found by the writers in Puget Sound bogs varies from 32.3 to 76.9 p.p.m., average of all being 50.4 p.p.m. Some of the latter results are in accord with those reported by ENDELL[4] for European bogs. BERGMAN implies that his field determinations were not exact, and it seems probable from statements made in his paper that all of the gases were not removed from the solutions analyzed in the laboratory. The amount of carbon dioxide found in the Puget Sound bogs is over 325 per cent greater than that found in the Minnesota bogs. This difference may have been caused by seasonal variations, higher temperatures of the Minnesota water, greater light penetration, and differences in regional flora conditions. In agreement with the writer's observations are those showing increases from the *Carex* stage to the *Chamaedaphne-Andromeda* and the *Larix-Picea* stages, and a further increase from these stages to that of the bog.

Since oxygen was entirely absent from the dissolved gases in five of the Puget Sound samples, and the maximum amount occurring in the other ten was only 3.2 per cent of the total gas dissolved, it would

[4] Jour. Prakt. Chemie. 82:414–422. 1910.

seem that oxygen deficiency must present serious difficulties. The growth of roots of any ordinary land plants, whose seeds might in any way be carried into the bog, or whose vegetative parts might begin to grow forward into the bog from any bordering association, would be either greatly retarded or entirely prevented.

The presence of methane in bogs that have developed by forming a floating mat on open bodies of water correlates with the well known fact that large amounts of methane are produced by the decay of masses of organic matter at some depth in undisturbed waters. It seems to follow naturally that the amount of methane would be greater in the deeper layers than in the upper, due to its formation and solubility. The presence of the methane decreases the partial pressure of the other gases, and thus reduces their solubilities. This is shown by the smaller amount of nitrogen, the least soluble of the gases, dissolved in the waters of the wet bogs, and by the high concentration of nitrogen in the gases liberated in Esperance Bog. The presence of methane may also correlate with the earlier stage of development found in the wet bogs. No work on the effects of this gas on the growth of roots has come to the attention of the writers.

Summary

1. The differentiation between wet bogs and dry bogs appears to be characterized by the gases dissolved in the waters of the bogs.

2. Wet bogs contain methane while dry bogs do not.

3. Wet bogs contain greater concentrations of carbonic acid than the dry bogs.

4. The presence of methane causes the liberation of nitrogen from solution, and thus wet bogs contain smaller dissolved quantities of this gas.

5. The oxygen content in all samples of bog water examined is practically nil. This marked oxygen deficiency when viewed in the light of the effects of such conditions on ordinary land plants, found by other workers, leads to the inference that this condition must be a large factor in the inhibition of non-bog plants from bogs of the Puget Sound region, and that bog plants are more tolerant of these conditions.

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DEVELOPMENT OF OVULE AND EMBRYO SAC OF COCOS NUCIFERA[1]

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(WITH THIRTEEN FIGURES)

Introduction

It seems strange that a plant so interesting and economically important as the coco palm has received so little attention morphologically. Our knowledge of the morphology is fragmentary and limited. The agronomy of the coco palm has been studied extensively; and lately the development of the inflorescence, the female flower, and the stony layer has been investigated (15). The present paper is intended to give only a description of the development of the ovule and the embryo sac at the time of fertilization, and later it is hoped to give a full account of fertilization and the development of the endosperm and embryo.

While, as might be expected, the results of this study do not show wide deviation from the usual series of events in monocotyledons, they have value in indicating the extent of variation within a given order. RADERMACHER (18), studying *Nipa fruticans*, found that the archesporial cell functions directly as the embryo sac mother cell, which divides into a "dyade." The chalazal cell enlarges and becomes the macrospore of the sac. The mature embryo sac possesses eight nuclei. This writer also investigated *Actinophloeus macarthurii* (*Ptychosperma macarthurii* H. Wendl.), and although his series is incomplete, found that the embryo sac mother cell is deep seated, with eight nuclei.

BAUCH (1) observed the 2-nucleate embryo sac of *Phoenix* with three complete degenerated cells as remains of the tetrad cells. According to this investigator *Licuala* has an 8-nucleate embryo sac, and *Sabal* and *Zalacza* possess a widened one. The development of the embryo sac of *Dypsis*, which is 8-nucleate, is similar to that of

[1] College of Agriculture, University of the Philippines, Experiment Station Contribution, no. 459.

Calyptrocalyx. He also saw the embryo sac of *Heterospathe*. *Nephrosperma* and *Verschaffeltia* have antipodals which remain long in the sac as an inward prominence of the nucellus. *Ptychococcus* and *Areca* show the remains of the tetrads in their embryo sacs, the former possessing only two, the latter three. BAUCH[2] states:

> The embryo sac mother cell is formed in the first and second row of cells of the nucellus. There may be also formed two embryo sac mother cells, but only one develops, the other degenerates. In *Cocos*, also, the remnants of the tetrahedral cells, which are located near the micropyle, are evident. It is therefore apparent that the cell which is near the chalazal develops into the primary embryo sac mother cell.

So far as the writers know, no work on the ovule or embryo sac of *Cocos nucifera*, except that of BAUCH cited by RADERMACHER (18), is on record.

Material and methods

The material used in this study was gathered between March and April, 1925, from twenty trees growing in the Bacomo Coconut Plantation of the College of Agriculture, University of the Philippines. Two of the trees were killed, and all unopened spadices in which the female flowers could be differentiated by the unaided eye were fixed. Twelve inflorescences were selected, and their dates of opening carefully noted. Inflorescences were gathered at intervals of 3, 4, 6, 9, 12, 15, 19, and 22 days respectively after they opened, until the stigmas were exposed. All female flowers from the inflorescences were then fixed. Slabs were cut from the two sides of the younger pistils and from the four sides of the older ones, and then fixed *in toto* with their perianth segments on or removed.

Only formo-aceto-alcohol (4) and formo-alcohol were used to kill and fix. After fixing the material was washed in 70 per cent alcohol, dehydrated by passing through successive grades of alcohol, dealcoholized in several ascending series of xylols, imbedded in paraffin, and cut with the Spencer rotary microtome into sections 5–7 μ. The appearance of large vacuoles in the sac and the presence of tannin idioblasts in the pericarp made cutting the material rather difficult. Flemming's triple stain was used in some cases. Haidenhain's iron-alum with Orange Gold dissolved in clove oil as background was used throughout, and proved to be very satisfactory.

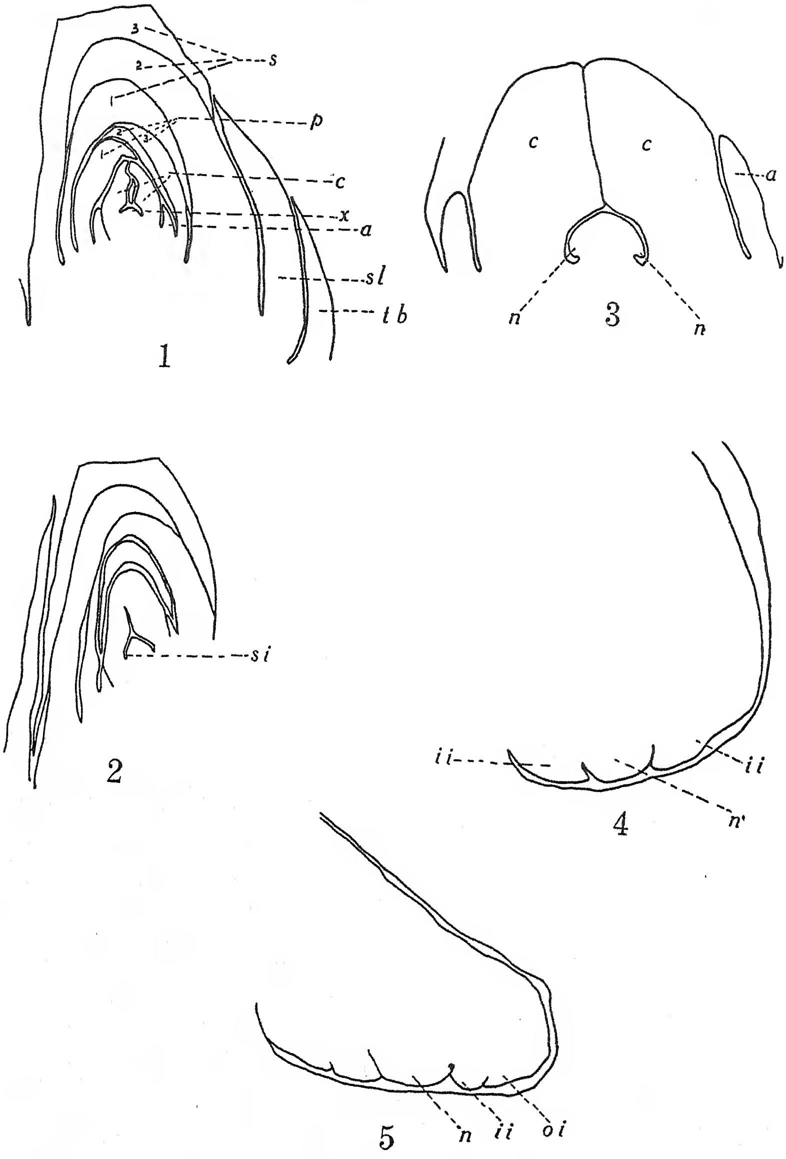
[2] Cited by RADERMACHER; original article of BAUCH not procurable.

Ovule

The development of the spadix and the floral segments of the female flower has been investigated (15). The centripetal appearance of the floral organs is as follows: (1) scaly leaves or "braceteoles," (2) sepals, (3) petals, (4) aril, and (5) carpels. When all the floral envelops and carpel walls have been formed and differentiated, the main axis of the flower starts as a papilla and commences to enlarge, gradually becoming prominent at the basal portion of the ovarial cavity (fig. 1). This protuberance consists of an epidermal layer of cells, most of which are squarish to rectangular, with their long axes tangential to the periphery. The hypodermal cells are isodiametric, filled with plenty of protoplasm and large nuclei. Their growth keeps pace with that of the carpel walls. As the main axis enlarges, the epidermal and hypodermal cells divide and elongate periclinally, and their cytoplasmic contents become less thick than those at the side. This gives the axis a pyramidal appearance (fig. 2). Simultaneously with the elongation of the cells at the apex, the basal cells at the sides (*s*) divide periclinally at first, and division rather than elongation is their first activity. By repeated periclinal divisions of the cells at the sides, and radial elongation and enlargement of the cells at the apex of the axis of the flower, the basal lateral sides are pushed into the three loculi of the carpel walls. These protuberances, which at first are slight, constitute the nucellus (fig. 3^n) of the ovules. By one-sided growth each primordium becomes bent toward the base of the ovary, developing into the anatropous ovules. It is interesting to note, however, that in the coco palm the usual development of the anatropous ovules as shown in *Lilium philaselphicum* (7) is not closely followed. The nucellus appears first as a few-celled papilla, with projections from which the integuments develop (figs. 4, 5).

The hypodermal cells of the nucellus then divide periclinally and anticlinally. The activity of that mass of tissue is responsible for the enlargement of the nucellus, rather than the epidermal. The epidermal cells remain squarish and become comparatively smaller than the hypodermal cells, which in their juvenile stages are nearly identical in size. Anticlinal division of the epidermal cells enables it to keep pace with the increasing nucellar tissue.

The integuments do not begin to appear until after the inflorescence has attained a maximum circumference of 15–18 cm. (meas-



FIGS. 1–5.*—Fig. 1, median longitudinal section of female flower showing tertiary bract (*tb*), scaly leaves or "bracteoles" (*sl*), sepals (*s*), petals (*p*), aril (*a*), carpels (*c*), and floral axis (*x*); ×11.5. Fig. 2, median longitudinal section of older flower showing pyramidal appearance of floral axis; *si*, sides of axis where nucellus of ovule is developed; ×11.5. Fig. 3, median longitudinal section of ovary showing beginnings of nucellus (*n*) of ovules, within carpel walls (*c*); ×53. Fig. 4, longitudinal section of anatropous ovule with inner integument (*ii*) already developed; ×235. Fig. 5, longitudinal section of much older ovule with two integuments, inner (*ii*) and outer (*oi*), appearance of latter being belated; ×235.

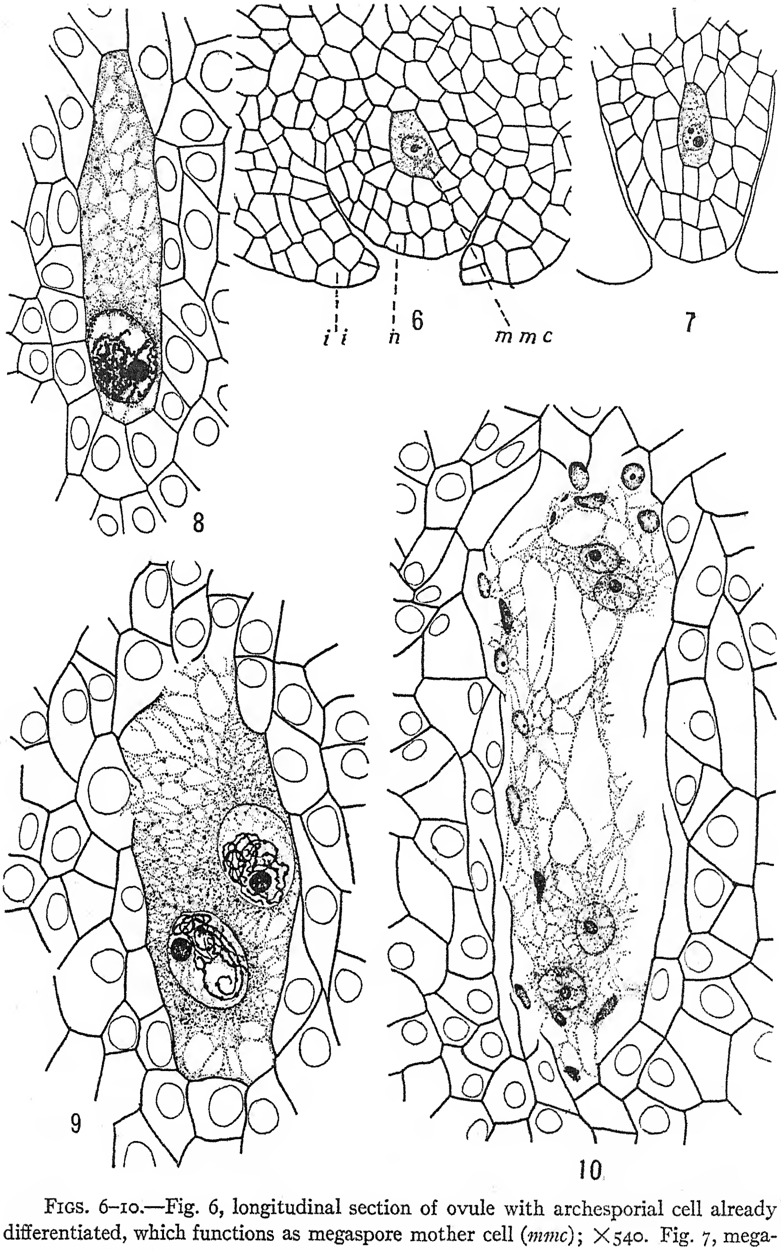
* All drawings were made with the Spencer microscope, Bausch and Lomb lucida, bar length 110, and the mirror at an angle of 55°.

ured with the inner spathe still attached). The inner integument (fig. 4) is the first to develop, followed by the outer (fig. 5).

Embryo sac

The ovule attains a considerable size, with its integuments fully differentiated before the archesporial cell becomes evident. The archesporial cell becomes the megaspore mother cell, and enters synapsis after the two integuments have long been evident. At first the megaspore mother cell is apparently undifferentiated from the surrounding cells, but soon it becomes grumous and is then readily distinguishable (figs. 6, 7). It can easily be recognized from the rest of the nucellar tissue by its size and great staining power. The presence of a large nucleus and its richness in cytoplasm make the megaspore mother cell more prominent. The megaspore mother cell is apparent after the inflorescence has emerged from the outer spathe, and while still enveloped by the inner spathe. It is more or less squarish, with the nucleus at the center of the embryo sac (fig. 6) to begin with, but soon elongates with its micropylar portion enlarged (fig. 7). The mother cell usually appears at the third layer of cells of the nucellar tip, but may sometimes be deep seated at the fourth or fifth layer. As the cell enlarges and elongates, there seems to be a definite migration of the nucleus to the micropylar end. Vacuolation of the one-celled embryo sac becomes apparent and progresses as the sac enlarges. The cytoplasm is denser toward the micropylar portion of the cell and vacuolated at the chalazal region. The writers have failed to find more than one archesporial cell developing, but BAUCH maintains that he found two, only one of which became functional.

The archesporial cell elongates and increases in size, always maintaining the bulbous and bulging appearance, wider at the micropylar end, with the chalazal end somewhat narrow. More granular bodies seem to accompany the enlargement of the nucleus. The vacuoles enlarge in the nucleus, and granular bodies appear in the network of protoplasm. Later, within the nuclear membrane a network of fine dotted threads is partially meshed by granular substance. The first stage which can be identified as indicating approaching division is the slender spireme (fig. 8). The threads become thicker and their dots larger, the nucleolus still remaining prominent. Before



FIGS. 6–10.—Fig. 6, longitudinal section of ovule with archesporial cell already differentiated, which functions as megaspore mother cell (*mmc*); ×540. Fig. 7, megaspore mother cell beginning to elongate; ×540. Fig. 8, megaspore mother cell at synaptic stage; ×890. Fig. 9, dinucleate stage of embryo sac; daughter nuclei at synaptic stage; note absorption of nucellar tissue beginning at chalazal end; ×890. Fig. 10, quadrinucleate stage of embryo sac, two nuclei at micropylar end and two at chalazal end; note destruction of nucellar tissue; ×890.

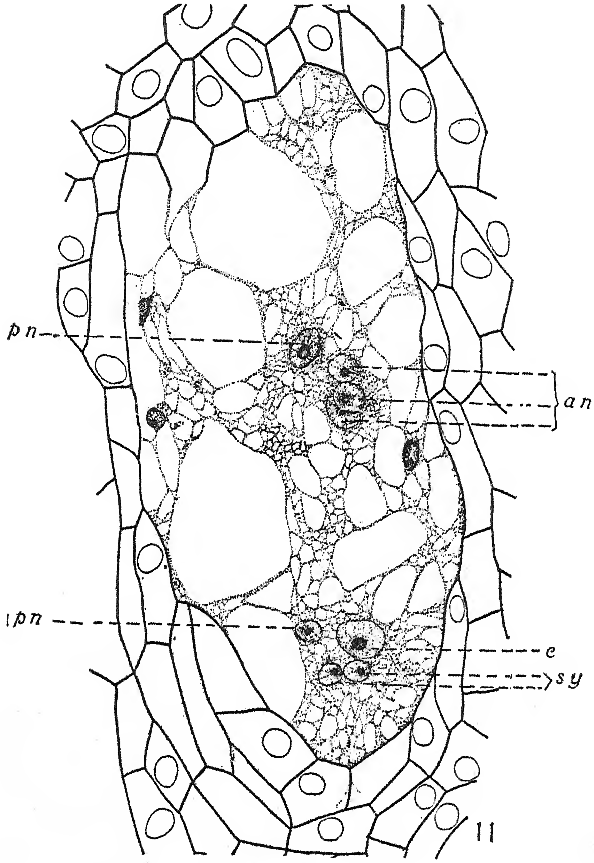
finally passing into the contracted state, the ribbon just described begins to close round the nucleolus. The nucleus now enters the period of synapsis, in which it remains for some time.

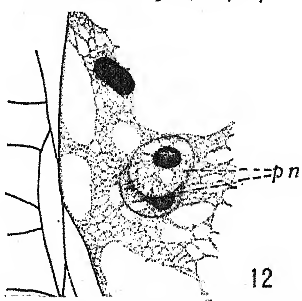
The nucleus of the megaspore mother cell of the embryo sac next divides into two, more or less unequal in size, without wall formation (fig. 9). This takes place before the emergence of the inflorescence from the envelope of the inner spathe. In the coco palm the formation of the tapetal cell and the four megaspores is eliminated, the two daughter nuclei lying side by side. They are vacuolated, and in the early synaptic stage of division. More granules are present in the cytoplasm of the sac. At this stage the sac shows signs of disintegration and absorption of the nucellar cells, which begins at the chalazal region (figs. 9, 10). Since the formation of the tetrad is eliminated, this first division of the megaspore mother cell must be heterotypic in nature. In the first division of the megaspore mother cell it was not possible to count the number of chromosomes.

At the binucleate stage of the sac no polarity is evident. Vacuolation begins at the micropylar and chalazal ends, and the daughter nuclei are at the center of the sac. Both chalazal nucleus and micropylar nucleus are commonly similar in shape and age, although the chalazal nucleus seems more advanced in development (fig. 9).

At the quadrinucleate stage of the sac polarity is in full display (fig. 10). The four daughter nuclei are at the ends of the sac, two at the micropylar and two at the chalazal end, the micropylar nuclei being larger than the chalazal. The two chalazal nuclei are unlike in size, the one near the center of the sac being slightly larger. The surrounding nucellar cells undergo disintegration, and their nuclei are freely liberated in the sac. Vacuoles are larger at the center of the sac, and cytoplasm thickest at the two ends. The nuclei apparently rest at this period.

When the sac reaches the octonucleate stage, it seems that the enlargement of the sac has gone far enough. All the chalazal cells show great depletion, almost to the micropylar end (fig. 11). This therefore makes the nuclei appear to be at the center of the sac. The micropylar group consists of three nuclei of the same size, one of which is the polar nucleus (*pn*). The larger one is the egg (*e*); the two similar ones are the synergids (*sy*). The chalazal group consists of





FIGS. 11, 12.—Fig. 11, octonucleate stage of embryo sac before final orientation of nuclei: *e*, egg; *sy*, synergids; *pn*, polar nuclei; *an*, antipodals; ×890. Fig. 12, fusion of polar nuclei; ×1060.

three congregated antipodals (*an*) and the elongated polar nucleus. It seems evident that the polar nucleus from the chalazal region is much larger than that from the micropylar region. The antipodals migrate to the chalazal pole, and before the fusion of the polar nuclei begin to disintegrate. The union of the two polar nuclei (fig. 12) is accomplished by the motility of the two nuclei approaching each other, and meeting and coalescing near the center of the embryo sac. The polar nucleus of the chalazal region apparently is at rest and the micropylar one appears more motile. This same condition occurs in *Luzula*, *Alisma*, *Carex*, *Triglochin*, *Orchis*, *Ornithogalum*, and *Nothiscordum* (**22**). Coalescence takes place before fertilization and before the pollen tube tip has reached the embryo sac.

At the final orientation of the component parts of the sac, it tapers at both ends and becomes more enlarged at the middle. This is not a general rule, however, as other shapes may also be round, depending on the amount of absorption and destruction of the nucellar tissues by the developing sac. Fig. 13 shows a mature embryo sac, at the chalazal end of which are the three antipodals, which are faintly recognizable. They are surrounded by thick protoplasm, and connection with the rest of the contents of the sac seems lost.

At the micropylar end is the egg apparatus, which is derived from the nuclei at the micropylar group. Prior to the migration of the polar nucleus, the egg is already differentiated from the two synergids in size (fig. 11). It becomes greatly vacuolated and enlarged, and is inserted somewhat lower down in the walls of the embryo sac.

A complete *Cocos* embryo sac is pictured in fig. 13. The antipodals (*an*) have gone into progressive degeneration. The endosperm nucleus (*en*) has assumed a central position, with a massive nucleolus, and with a spherical form. The egg apparatus, consisting of a very prominent egg (*e*) and two synergids (*sy*), has approached maturity. At this stage the synergids begin to show signs of degeneration.

In the development of the ovule and embryo sac of *Cocos nucifera* much time is involved The internal growth of the axis of the flower must have begun long before the inflorescence has emerged from the outer spathe. The ovules differentiate and attain their natural position when the inflorescence has escaped from the outer spathe and

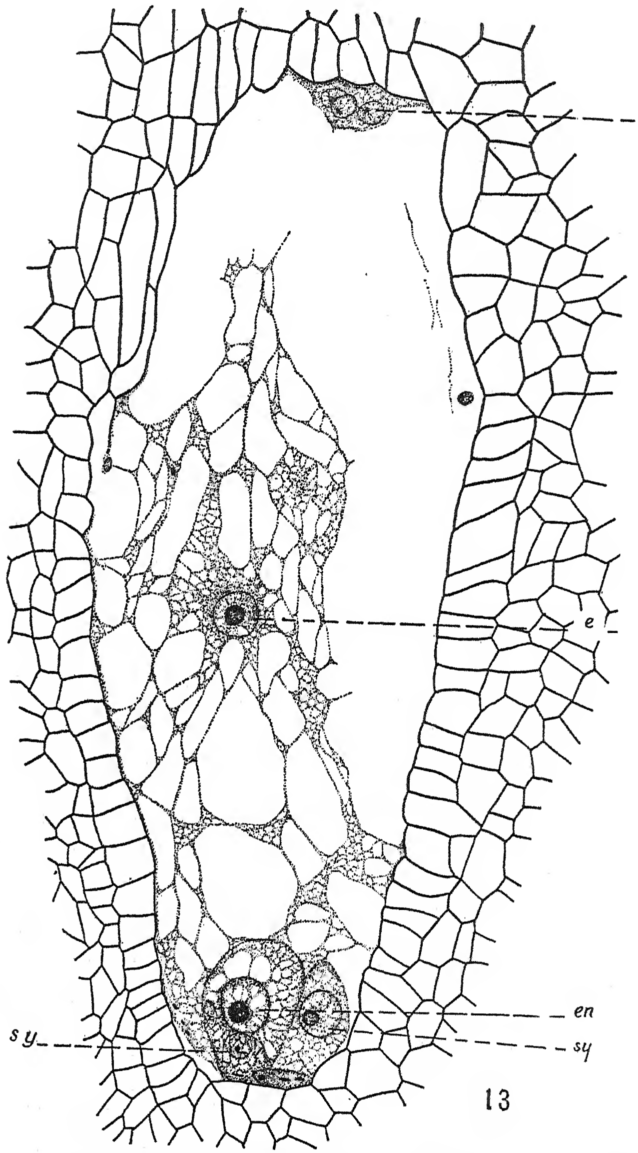


FIG. 13.—Mature embryo sac showing antipodals (*an*) at process of degeneration, endosperm nucleus (*en*) already formed, and synergids (*sy*) of egg apparatus at stage of degeneration; ×540.

made appearance from the axil of the clasping petiole. At this time the integuments are already formed.

INFLORESCENCE	MEGASPORANGIUM AND OOGENESIS
Inflorescence still inside of petiole, with outer spathe and inner spathe; 52 cm. long, and 15.3 cm. circumference*	Axil of the pistil already differentiated, bulging and initiation of ovules
Inflorescence out of outer spathe; circumference 14.3 cm.	Anatropous situation of ovules prominent
Inflorescence out of outer spathe; circumference 15.8 cm.	Outer and inner integuments formed
Inflorescence out of outer spathe; length 40 cm., circumference 18 cm.	Archesporial cell differentiated
	First division of megaspore mother cell
Inflorescence out of outer spathe; length 66.5 cm., circumference 27.2 cm.	Second division of megaspore mother cell
	Third division of megaspore mother cell
Just after opening of inflorescence	Fusion of polar nuclei and disintegration of antipodals
Emergence of stigmas (in receptive condition)	Disappearance of synergids and enlargement of egg and endosperm nucleus

* Length measured from base of lowest rachilla to tip of spathe; circumference based at largest portion of spadix.

Discussion

It has been the constant attempt of botanists to unravel, by a comparative study of the development of their gametophytes, that complicated fabric of phylogenetic relationships existing among plants. Unless a thorough study of nearly all the species known is carried out, no fair comparison can be made. It must be understood, however, that it is not within the scope of this investigation to offer any solution of this question.

The female flower arises as an emergence in the axil of the tertiary bract of the rachilla. At the axil of the tertiary bract and the floral cone, small papillate protrusions appear, which elongate, bend, and cover the juvenile flower. As soon as these scale leaves develop,

the three sepals appear simultaneously from the basal portion of the flower primordium, one overlapping the other at the apex. The petals, alternating with the sepals, next emerge simultaneously, first as rudimentary papillae, and soon differentiating like the sepals. The next to the last structure to emerge is the aril. The carpel walls are the last to develop.

The ovules may be considered cauline, as they arise from the central axis of the flower. In *Balanophora* and *Loranthus* (7) several writers found a similar situation, where a structure (or "mamelon") arises at the bottom of each sporangial chamber and grows until it completely fills it. *Rhopalocnemis phalloides* exhibits similar growth of the floral axis, especially before the appearance of the archesporium. The floral axis of *Cocos nucifera* enlarges, and toward each loculus is produced a papilla which serves as nucellus of the ovule. In other words, the nucellus of the nascent ovule is directed toward the receptacle. This case, where the usual development of the anatropous ovule is not followed, is an exception to the rule. The integuments are two, inner and outer.

Only one cell constitutes the archesporium, which according to our observation functions as a megaspore mother cell, and develops into the sac direct. According to RADERMACHER, *Nipa fruticans* also possesses an archesporium of one cell.

The cells of the archesporium, whether they be one or more, may by transverse division give rise to a primary parietal cell and a primary sporogenous cell. In *Cocos*, however, the archesporium, which is one-celled, never cuts any parietal cell. COULTER and CHAMBERLAIN (7) quote that *Avena fatua*, *Allium*, *Hemerocallis*, *Lilium*, *Erythronium*, *Tricyrtis*, *Sisyrinchium iridifolium*, *Gymnadenia conopsea*, *Orchis pallens*, and also *Commelina stricta* and *Iris stylosa* (10) do not develop any parietal cell. *Canna indica* (10) sometimes develops a parietal cell and sometimes does not.

The archesporial cell functions directly as the megaspore mother cell similar to *Lilium*, *Fritillaria*, *Funkia*, *Tulipa*, *Convallaria* (23), and *Erythronium* (19). Among the primitive aquatic plants, COULTER and CHAMBERLAIN give *Typha* and *Alisma* as exhibiting similar development of the megaspore mother cell. HALL (11) cites *Limnocharis* as possessing parallel development of the megaspore mother

cell. CALDWELL (3) reports that the mother cell does not divide in *Lemna*. Among the higher families *Narcissus* (10) and *Costus* (12) possess an undividing mother cell. *Nipa* (18) also shows an undividing megaspore mother cell, similar to that of *Cocos*.

BAUCH stated that a sign of degeneration of megaspores was found by him. Unfortunately the original article of BAUCH could not be obtained, so a fair comparison could not be made. However, judging from his statement[3] he seems to believe that the megaspore mother cell must have divided to form the two daughter megaspores, the chalazal becoming functional. This is contrary to what is reported in this paper. This discrepancy and divergence of interpretation is not uncommon; the case of *Richardia africana*, where MICHELL and GOW (16) seem not to agree as to the origin of its sac, is an example of differences of this kind. GOW found its origin to be a duplicate of *Cocos nucifera*, but MITCHELL found that four megaspores were produced. We base our conclusion on observations in the examination of hundreds of slides, and believe that the production of the megaspores or the tetrad in the normal development of the sac is entirely suppressed. Signs of disintegration of the megaspores were observed by BAUCH at the micropylar region of the sac. In many of our preparations we have found remains of nuclei from the nucellar cells undergoing absorption by the developing sac at the micropylar end (fig. 10). Two, three, or more nuclei may be crushed together and stain deeply so as to give an appearance of distintegration, or what BAUCH might have termed daughter megaspores experiencing disintegration or degeneration. These nuclei are plentiful, especially at the quadrinucleate stage of the sac.

The development of the megaspore mother cell into the embryo sac direct is not only found among the monocotyledons, but also in the dicotyledons. COOK (6) on *Rhytidophyllum*, CHAUVEAUD (5) on *Vincetoxicum*, YOUNG (24) on *Melilotus alba*, DASTUR (8) on *Hydnora africana*, and D'HUBERT (9) on *Opuntia* report cases of undividing mother cells. *Peperomia*, *Piper*, and *Heckeria*, as mentioned by JOHNSON (13, 14), also develop undividing mother cells.

The regular division from the megaspore mother cell (or 1-nucleate stage of the embryo sac) to the octonucleate sac, follows the usual

[3] Cited by RADERMACHER.

situation common in *Calopogon* (17), *Epipactis* (2), and in *Epidendrum variegatum*, *E. cochleatum*, *E. verrucosum*, *E. globosum*, *Coelogyne massangeana*, *Pogonia macrophylla* (20), and others.

The early degeneration of the antipodals, previous to the fusion of the polar nuclei, seems to indicate that they are ephemeral and do not persist long, as in *Nephrosperma* and *Vershaffeltia* (1). Typhaceae, Naiadaceae (*Potamogeton*), Alismaceae, Pontederiaceae, Liliaceae (except *Ornithogalum*), Scitamineae, and Orchidaceae (10) show ephemeral antipodals.

It is evident, as with *Eichornia* (21) and *Lilium* (7), that the fusion of the polar nuclei forms the endosperm nucleus.

Summary

1. The axis of the flower must have developed long before the emergence of the inflorescence from the outer spathe, and develops the ovules after it has escaped from the spathe. The development of the embryo sac continues and proceeds up to about the receptive stage of the stigma.

2. The ovules are cauline, arising from the central axis of the flower. The floral axis bulges out, and papillae which serve as the nucellus of the nascent ovules are pushed toward each loculus of the ovary. The inner and outer integuments develop in succession.

3. The archesporium is one-celled, which does not cut off any parietal cell, but functions directly as the megaspore mother cell, which develops the embryo sac.

4. The megaspore mother cell divides in the usual manner, and produces the octonucleate sac, hence upon maturity we have the egg, two synergids, two polar nuclei, and three antipodals.

5. The polar nuclei fuse just after the opening of the inflorescence and before the degeneration of the antipodals and synergids. The polar nuclei migrate toward the center of the sac, where they fuse.

6. The synergids begin to disappear just before the time when the ovary becomes receptive.

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REVEGETATION OF A DENUDED TROPICAL VALLEY

Duncan S. Johnson

(WITH FIVE FIGURES)

On November 8 and 9, 1909, the Blue Mountain region of Jamaica experienced an unprecedented rainfall of 27 inches, 18 inches in one 24-hour period at Cinchona. This caused disastrous floods, with great loss to the coffee plantations and to the vegetable gardens of the negro planters in the hills. Scores of acres of coffee fields were stripped to the bare rock, and even native forest was torn from the higher mountain sides, where the rainfall was probably heavier yet, and washed down the rivers to the sea, a mile below and a dozen or more miles away.

The valley of the Cascade River (fig. 1), which drains part of the southern slope of Mossman's Peak in the Blue Mountain Range, offers an excellent example of the havoc wrought by this flood. Six years and three years before the flood (1903 and 1906) the writer had crossed this valley near the 3000 ft. level; on the way from Cinchona to Blue Mountain Peak. It was then covered by a practically continuous forest, composed of many species of dicotyledonous trees with abundant shrubs and lianas. Shreve (4) in a similar forest nearby noted, besides occasional species, 33 species of characteristic trees and shrubs, 12 lianas, and 34 ferns and herbaceous angiosperms.

In 1910 I again crossed this valley at the same level, and was startled to see how completely the flood had destroyed the forest seen there in 1903 and 1906, as well as the coffee fields and native forest higher up. Now, six months after the flood, the coffee trees were gone from many acres of the mountain side, the buildings of the Whitfield Hall coffee works had been crushed by a landslide from the hill above, and the débris from this and from landslides in other gullies was washed down to help bury parts of the valley floor below. A photograph made in 1926 showed nothing in sight at the works but one corner of the cement drying floor or "barbecue." All but this

small corner of the barbecue was covered by detritus from a cliff above, and now, after sixteen years, it is overgrown by cane and bushes and young trees that flourish in the seepage from a water conduit that formerly supplied the water wheel at the coffee works.

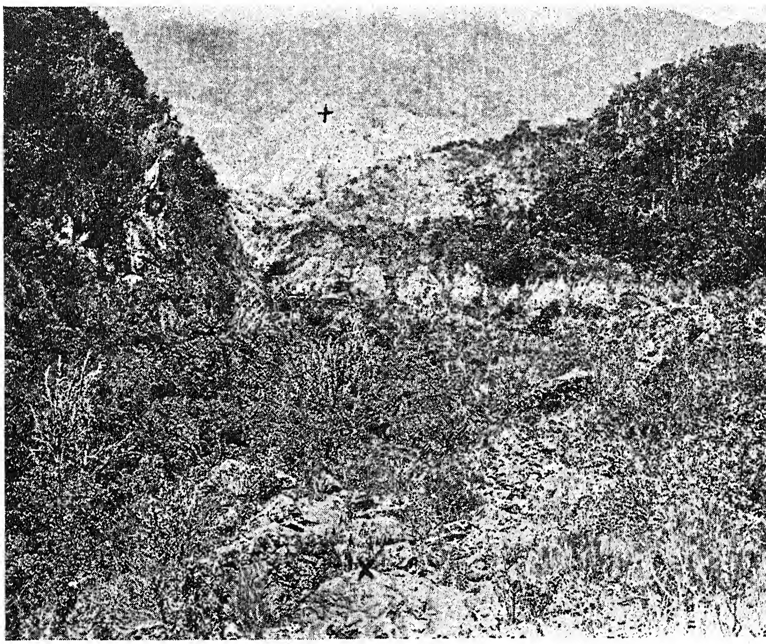

FIG. 1.—View looking up Cascade Valley over area studied, showing *Arundo*, *Baccharis*, *Myrica*, *Piper*, *Vernonia*, and *Rhytidophyllum* (at *X*) photographed June 1926, when clouds covered the large washout; cf. Johnson (2) fig. 2, noting in both the points here marked *o* and +.

The floor of the valley, 500 yards below the works, where I crossed it for the third time in 1910, was now a desert-like waste of bowlders and gravel for the whole 200 yards of its width (1). It was not merely the herbs and shrubs of the forest floor that were gone, but the large trees with their covering of epiphytes and lianas had disappeared completely. The only plants seen in this stony waste in 1910, across the whole width of the valley where our trail crossed it, were very scattered seedlings of the Papaveraceous shrub *Bocconia frutescens*, or celandine, and far fewer seedlings of half a dozen other

dicotyledons common on the surrounding hills. No lichens were seen, and no algae noticed save in the stream on the western side of the valley.

In July 1919 I again crossed the Cascade by the same trail, and was surprised to find how slowly the vegetation was recovering the waste area (2). Even then, nine and a half years after the flood, nine-tenths of the surface of the soil of this area was still bare rock and gravel. Relatively few of the many species growing abundantly on the surrounding hills had succeeded in becoming established in this rubble-like, well drained soil. A census of ferns and seed plants made at this time showed the presence in the formerly denuded area of seven ferns, of which *Gymnogramme tartarea* and *Pityrogramme calomelaena* were rather frequent, while *Trismeria trifoliata* was represented by dozens and *Pteris longifolia* by scores of plants. The other three ferns were rare. Many of these ferns were then so large (half a yard high or more) that it hardly seemed possible that they could have arisen from spore and prothallus in nine years. However, the absence of any sign of these ferns in 1910 and the fact that each clump of ferns seen in 1919 consisted of but one or two branches and leaf clusters, seemed to indicate that each fern plant had arisen where it stood from a prothallus. The sole monocotyledon seen, the cane *Arundo*, was confined to borders of the two branches of the stream, which join a little below the area. Of the twenty-one dicotyledons found, ten were Compositae, three were Asclepiadaceae, and two were Verbenaceae. More than half of them (14) were woody.

At that time *Vernonia permollis* was clearly the predominant plant of the area. Clumps ranging in size from seedlings up to plants 2 yards high were scattered throughout, but chiefly in definite although rather crooked rows along the minor gullies which drain the raised region between the main stream and a side branch entering from the east. After *Vernonia permollis*, *Bocconia frutescens* (then 2 or 3 yards high), *Solanum torvum*, and *Vernonia acuminata* (the two latter up to 2 yards high) were the most prominent plants scattered over the gravelly valley bottom.

The fifteen remaining dicotyledons, represented by from half a dozen to a dozen or two each, included species of *Piper*, *Pilea*, *Iresine*, *Begonia*, *Asclepias* (2 spp.), *Philibertella*, *Duranta*, *Verbena*, *Solanum*, *Maurandia*, *Ageratum* (2 spp.), *Mikania*, *Eupatorium*, *Bac-*

charis (a dozen or two plants), *Pluchea*, and *Bidens*. *Pilea* and *Bidens* were most numerous of all these.

Encrusting the surfaces of the pebbles and bowlders among which the dicotyledons grew was found the most abundant and constant species. This is the Chroococcaceous alga *Gloeocapsa magma*, which often forms velvety crusts half a foot square on the otherwise naked rocks that are of a rather bright maroon when moist but chocolate brown when dry. No lichens or mosses were noted in 1919.

In July 1926 I visited the Cascade Valley for the third time since the flood. The region along the trail is still sparsely covered with vegetation, perhaps a quarter to a third of the surface of the area being shaded by plants. Although the number of individual plants has not yet become sufficient to cover the ground, a number of new and interesting species have appeared.

Gloeocapsa still reddens large areas of pebble and bowlder surface across the whole width of the valley (fig. 2). Lichens are still so inconspicuous that Professor PLITT (in July 1926) at first thought them lacking. Further search revealed a very sparse lichen population, including altogether 18 species, most of them represented by only 5 or 10 specimens seen. On the rocks grew the crustose *Buellia* sp.? with the foliose *Leptogium* sp.?, *Parmelia perlata*, *P.* sp.?, *Anaptychia hypoleuca*, and *Coccocarpia pellita*. On the rocks also were found the fruticose *Stereocaulon cornutum*, *S. ramulosum*, and *Usnea hirta*. On the soil are *Cladonia pycnoclada* and *C.* sp.?. The bark of the shrubs bore *Haematomma puniceum*, *Lecanora subfusca*, *Parmelia perlata*, *Theloschistes flavicans*, *Usnea hirta*, and *U. rubigena*. Of all these only the *Buellia*, *Haematomma*, and *Lecanora* could be found in any quantity. *Usnea hirta*, although not numerous, is conspicuous because of its size. It is the sparseness rather than the presence of the lichens here that was most unexpected. But the persistence and even increase of *Gloeocapsa* on these hot dry rocks is very surprising, especially when we realize that it must often be exposed to a scorching tropical sun for many hours daily, and may be without rain for days or rarely for several weeks together. There is a copious dew in the valley each clear night, however, while on cloudy nights, as was often the case in 1926, the fog settled down to condense on the rocks and plants of its floor.

The total number of species of mosses, ferns, and seed plants observed in 1926 was forty-one. The rarity of mosses and the complete absence of liverworts from the new vegetation of this area is in striking contrast with their prominence as pioneers in burned-over tracts on the neighboring Blue Mountain Peak, a prominence they commonly have also in burned tracts in the temperate zones.

FIG. 2.—Group of fruiting plants of *Psilotum* growing between small bowlders; note young, simple shoot of this at left of knife; *Trismeria* at left and at extreme right *Gloeocapsa* on bowlders at right and below; (handle of pocket knife shown is 4 in. long); photographed June 1926.

Only one species of sterile moss was seen in 1926. Of pteridophytes, nine species are now established. Of these *Trismeria trifoliata* is most abundant (fig. 2). *Dryopteris oligophylla*, *Pteris longifolia*, and *Gymnogramme tartarea* are more moderately and about equally abundant. The pteridophyte I was most surprised to find last summer in this still desert-like habitat is *Psilotum nudum*, which had hitherto been seen but rarely in Jamaica and then always growing as an epiphyte. Here on the dry ridges and slopes of this gravel bed I found last summer over two dozen tufts of this *Psilotum*, growing sometimes in the scant shade of *Baccharis scoparius*, while else-

where, in the full sunlight, it grew out from between quite large bowlders (figs. 2, 3). How the rootless, humus-loving *Psilotum* can find adequate nutriment among these bowlders it is hard to guess. Its common occurrence here in this sort of substratum suggests at least something of the endurance of this archegoniate which has recently been regarded by KIDSTON and LANG as the most primitive of all living vascular plants.

Of the seven monocotyledons seen in the Cascade Valley last summer, the cane of the stream borders, *Arundo saccharoides*, is still by far the most abundant. Tufts of two smaller grasses, *Andropogon virginicus* and *A. gracilis*, are now scattered over the drier parts of the area, and two species of *Carex* accompany the grasses, although in smaller numbers. Quite unlooked for as an immigrant to the valley, at this stage of revegetation, was *Bletia verecunda*, which now grows freely, often two or three tall flower stalks together (2 or 3 feet tall), in the dry gravel and between the smaller bowlders. This orchid seemed to be flourishing in spite of the rapid drainage and the lack of humus; twenty plants were visible in one photograph. One vascular epiphyte *Tillandsia* (*setacea*?) was represented by two specimens seen, one on *Myrica*, the other on *Baccharis*. These two are the slender advance guard of this xerophytic epiphyte which will ultimately beset the trunks and branches of most of the woody plants of this area.

The dicotyledons are still, in spite of the recent invasion by the monocotyledons just mentioned, decidedly the dominant plants of this area, as they have been from the beginning. The two species of *Vernonia* and *Bocconia*, however, have now yielded the dominant place to two later comers, *Baccharis scoparia* and *Dodonaea angustifolia* Sw. (fig. 3). *Baccharis*, which was represented by but a few dozen small specimens in 1919, is now found in hundreds. These range in size from small seedlings to bushes 7 ft. high, with 2.5–3 inch trunks, and the fallen twigs of these plants are contributing as largely as any species to the scanty humus that is slowly accumulating between the pebbles. *Dodonaea* was rare and small in 1919, but it is now represented by dozens of plants, some few of them 8 ft. high with 1.5–2 inch trunks. These two shrubs now form the major part of the upper story of the vegetation. *Pilea microphylla* and *Micro-*

FIG. 3.—View of portion of area just below trail crossing, showing *Andropogon virginicus* (left below), *Baccharis* (left), *Pteris* (center above small card), *Dodonaea* (right between cards), and four colonies of *Psilotum* (each before a card) growing between pebbles strewn with fallen branchlets of *Baccharis* (card at left is 6.5×9.5 in.); photographed June 23, 1926.

meria viminea are present in scores, but are relatively inconspicuous because of their small size and their yellowish or reddish color. *Clusia havetioides*, common in forests at this level, as a *Ficus*-like epiphyte, has now populated the area with about 25 young specimens growing *on the soil*, often three or four in a group.

Perhaps the most striking woody dicotyledon is *Philibertella clausa*, an Asclepiadaceous vine with woody stems an inch in diameter and sometimes 100 ft. long, which sprawls in serpentine coils over many square yards of the warm gravel.

The remaining thirty-three dicotyledons are relatively scarce, and none of them is prominent except four or five plants of *Myrica microcarpa* just at the edge of the area and near the middle of the valley. These have now grown to trees with 4 or 5 inch trunks and crowns 10–12 ft. high but 15 ft. broad. Seen from the hillside above, their dark green foliage stands out against the prevailing gray green foliage and gray brown gravel.

At each visit to the Cascade Valley since the flood I have been surprised at the slowness with which the vegetation is recovering this virgin soil in a region where temperature, light, and rainfall are quite adequate for a rather dense forest. While it was assumed in 1910 that certain humus-inhabiting plants would be slow in settling on this new soil in the Cascade Valley, it was believed that a considerable number of the less exacting lichens, liverworts, mosses, ferns, and angiosperms from the neighboring hills would prove capable of settling there rather promptly.

There are then two important respects in which the course of repopulation of the valley floor has been surprising. The first of these is the slowness with which a complete covering of vegetation is developing. This is true in spite of the fact that 20 species of thallophytes and 41 species of cormophytes have, up to the present, established themselves on this area and are ready to cover it. Cursory observations were made in 1910 and 1926 of the revegetation of the floor of the Mabess Valley on the north side of the Blue Mountain Range, and at approximately the level of the Cascade area. These showed that here, with a similar average temperature, although a less varied one, with a greater rainfall and a higher humidity, the rocks along the stream that were bare in 1910 (just after the flood)

TABLE I

PLANTS FOUND IN CASCADE VALLEY IN 1910, 1919, AND 1926

Species	1910	1919	1926
ALGAE			
Gloeocapsa magma (Breb.) Kütz	....	×	×
Nostoc sp	....	....	×
LICHENS			
Anaptychia hypoleuca	....	....	×
Buellia sp	....	....	×
Caloplaca sp			×
Cladonia pycnoclada exalbercens	....	....	×
Cladonia sp	....	....	×
Coccocarpa pellita	....	....	×
Haematomma puniceum	....	....	×
Lecanora subfusca	....	....	×
Leptogium sp	....	....	×
Parmelia perlata	....	....	×
Parmelia sp	....	....	×
Ramalina sp	....	....	×
Stereocaulon cornutum	....	....	×
Stereocaulon ramulosum	....	....	×
Theloschistes flavicans	....	....	×
Usnea hirta	....	....	×
Usnea rubigena	....	....	×
ARCHEGONIATAE			
Moss (black, tufts sterile)	....	....	×
Adiantum tenerum Sw.	....	....	×
Aneimia adiantifolia (L.) Sw.	....	×	....
Blechnum occidentale L.	....	×	×
Dryopteris oligophylla Maxon	....	×	×
Gymnogramme tartarea (Sw.) Desv.	....	×	×
Pityrogramme calomelaena (L.) Link	....	×	×
Pteris longifolia L.	....	×	×
Trismeria trifoliata (L.) Diels	....	×	×
Psilotum nudum (L.) Gris.	....	....	×
MONOCOTYLEDONEAE			
Andropogon gracilis Spr.	....	....	×
Andropogon virginicus L.	....	....	×
Arundo saccharoides Gris.	×	×	×
Bletia verecunda R. Br.	....	....	×
Cyperus sp. (foliaceous bracts)	....	....	×
Cyperus sp. (small, scattered tufts)	....	....	×
Tillandsia setacea Sw.	....	....	×
DICOTYLEDONEAE			
Acacia ? (seedling)	....	....	×
Ageratum conyzoides L.	....	×	×
Ageratum houstonianum Mill.	....	×	....
Asclepias curassavica L.	....	×	....
Asclepias nivea L.	....	×	....
Baccharis scoparia Sw.	....	×	×
Arenaria lanuginosa (Michx.) Rohrb.	....	....	×
Begonia accuminata Dryand.	....	×	....
Bidens incisa Ker.	....	×	....
Bocconia frutescens L.	×	×	....
Cecropia peltata L.	....	....	×
Clusia havetioides Planch. et Tr.	..	....	×
Crotalaria striata DC.	....	....	×
Daucus carota L.	....	....	×
Dodonaea angustifolia Sw.	....	....	×
Duranta plumieri Jacq.	....	×	....
Eupatorium triste DC.	....	×	×
Gesneria exserta Sw.	....	....	×
Helosciadium leptophyllum DC.	....	....	×
Iresine celosoides L.	....	×	....
Lantana odorata L.	....	....	×
Lantana stricta Sw.	....	....	×
Lisianthes longifolius L.	....	....	×
Maurandia scandens A. Gray	....	×	×
Metastelma sp. ?	....	....	×
Micromeria viminea (L.) Urban	....	....	×
Mikania scandens L. (Wild.)	....	×	?
Myrica microcarpa Benth.	....	×	×
Philibertella clausa (Jacq.) Vail	....	×	×
Pilea microphylla L. (Liebm.)	....	×	×
Piper sp.	....	×	×
Pluchea odorata L. (Cass.)	....	×	×
Rebulnium hypocarpium Endl.	....	....	×
Rhytidophyllum tomentosum (L.) Mart.	....	....	×
Senecio discolor (Sw.) DC	....	×	×
Silene ?	....	....	×
Solanum torvum Sw.	....	×	?
Verbena bonariense L.	....	×	?
Vernonia acuminata Less.	....	×	×
Vernonia permollis Gleason	....	×	×
Viburnum villosum Sw	....	....	×

had by 1926 become completely covered by vegetation (figs. 4, 5). It seems clear that one chief preventative of the more rapid and complete reoccupation of the Cascade Valley by vegetation is the physi-

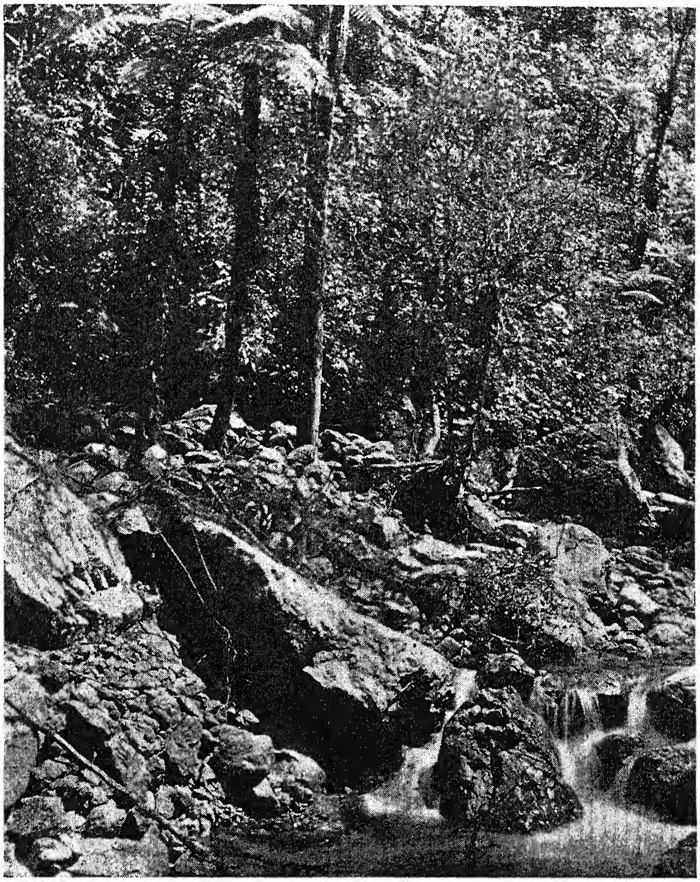

FIG. 4.—Banks of Mabess River (headwaters) taken June 1910, showing bare zone on each bank scoured clean by great flood of 1909; trunks of *Alsophila* with rosettes of *Caraguata* in background.

cal (rather than chemical) character of the shifting, well drained soil. On the other hand, it is evident that the considerably smaller and less evenly distributed rainfall, and the more constant sunshine of the Cascade Valley, as compared with the Mabess, has retarded the development of a complete covering of vegetation in the former.

The second surprising feature of the revegetation is the relative time of appearance of the several types of plant immigrants in this

FIG. 5—Banks of portion of same stream, photographed July 1926, showing stream border recovered with vegetation down to normal high water level; in background are *Alsophila swarzii*, *Cyathea* sp.?, *Heliconia bihai* (L.) Sw., *Geonoma swarzii*, and *Lobelia* sp.?.

virgin soil. *Gloeocapsa magma* is the only thallophyte that appeared early (in this case on the rock surface). The lichens and mosses which WARMING (6) states are the first invaders of fresh soil, are only just now, after sixteen years, becoming at all evident in the area, when a considerable covering of as yet quite open communities of vascular plants has already become established. This is clearly true in spite of the fact that the Cascade Valley has a true forest climate, of which, as TANSLEY and CHIPP (5) state, "in a forest climate a new bare soil tends to be first occupied by the lower forms of plant life such as algae, lichens, and mosses." These usual pioneers, as they go on to state, are commonly followed in the order here named, by annual flowering plants, perennial herbs (including grasses), and these still later by shrubs and trees. In the Cascade area, on the contrary, the first plants evident were dicotyledonous shrubs and half-shrubs, for example, *Bocconia*, *Vernonia*, *Baccharis*, *Dodonaea*, *Philibertella*, *Solanum*, *Mikania*, *Eupatorium*, and seven others; while ferns and the herbaceous angiosperms were slow in appearing. Even now, after sixteen years, out of fifty-eight vascular plants established in this area nine are perennial ferns, seven are perennial monocotyledons; and of forty-two dicotyledons only five are annuals, while six are herbaceous perennials, and the remaining thirty-one are shrubs or trees. It will thus be seen that in this tropical valley the whole order of immigration and establishment described by WARMING and by TANSLEY and CHIPP has been almost completely reversed. Whether this inverted order of establishment is generally characteristic of tropical regions must be determined by further observation. It is certainly surprising that the lichens, which in general need plenty of light, should be so slow in settling on the fresh rock surfaces of this valley. The absence of xerophytic bryophytes is also very unexpected and as yet unexplained.

It is hoped that further data concerning the progress of the revegetation of this valley, including something of its seasonal aspects, can be gathered during the coming decade or two. The preceding account may serve as a picture of the vegetation already established there in 1926.

Acknowledgment is here made to Professor C. C. PLITT for information embodied in this note concerning the lichens of this area

and to Dr. N. L. BRITTON, Dr. A. S. HITCHCOCK, and W. R. MAXON for the identification of dicotyledons, grasses, and ferns respectively. M. S. CURTLER aided by collecting the vascular plants of the area studied.

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MEIOTIC IRREGULARITIES IN A GIGAS FORM OF POTENTILLA ANSERINA[1]

MURIEL V. ROSCOE

(WITH PLATE X AND TWO FIGURES)

Methods and material

The buds for this study were collected on warm days, cut with a sharp razor, placed in chromo-acetic (0.75 per cent) solution and pumped. Fixation was followed by thorough washing in water and bleaching in a 10 per cent solution of a concentrated solution of sodium chlorate in hydrofluoric acid. They were then imbedded in nitrocellulose, and 5 and 10 μ sections cut. These were stained with Haidenhain's haematoxylin, and studied with a 1.5 mm. Zeiss objective and no. 12 ocular.

A "*grandis*" form of *P. anserina* L.[2] collected in Cape Breton, Nova Scotia, was featured, in addition to its *gigas* development, by pollen sterility and lack of fruit formation. Fig. 1 is a photograph of the aberrant and the normal forms, and shows clearly the vegetative luxuriance of the former. In contrast to the normal *P. anserina*, the "*grandis*" form is not found in the regular salt marsh, but at beach heads, where it gets the wash of the salt water but rarely.

Cytological observations

Late prophases or diakinesis stages show variable numbers of chromatin units. The variation in these numbers is probably due to a loose union of the paired or bivalent chromosomes. LONGLEY (18) found in some specimens of *Tripsacum* both univalent and bivalent chromosomes, which "makes the assigning of a definite haploid chromosome number difficult." Similar difficulty has been experienced in this form of *Potentilla anserina*.

[1] Contribution from the Laboratories of Plant Morphology, Harvard University.

[2] According to FERNALD's revision of the representatives of *Potentilla anserina* in Eastern America (7), the variety *grandis* T. & G. is included in *P. pacifica* Howell. The form under discussion, on account of its pubescence, is considered a "*grandis*" form of *P. anserina* L.

The plate shows division figures for this form. Metaphases are rarely regular, and figs. 3 and 4 are representative of the differences in chromosome number and degree of pairing. True metaphase plates are never formed. The chromosomes are distributed to the poles irregularly, and the process is featured by a number of laggards, some of which become left outside at interkinesis. The latter are univalents, and a portion are always resolved into micronuclei (fig. 5).

FIG. 1.—*P. anserina*, photograph of the *gigas* and normal forms

The unequal distribution is a cause for further abnormalities in the homotypic division. Fig. 6 shows an extremely irregular metaphase. The spindle on the left has received 22 chromosomes, which are very diverse as to size and arrangement. The spindle on the right contains a much smaller number, whose arrangement is indicative of abnormality.

Homotypic anaphases are abnormal (fig. 7), and in the majority of cases lead to a condition of polycary. An abnormality with three large nuclei of uncertain origin (fig. 8) is frequently seen, and undoubtedly is attributable to the hybrid constitution of the parent.

A drawing of a group of cells showing the end product of the second division is shown in fig. 2, and serves as a forceful illustration of polycary as a resultant of irregular chromosome distribution. The

cytoplasm of all the pollen mother cells during division is much vacuolated, and its appearance suggests degeneracy. The reduction division of this *gigas* form of *Potentilla anserina* is thus featured by abnormalities of various sorts, and it is not surprising that high degrees of pollen sterility result. In a majority of the mature loculi there is complete absence of cytoplasmic grains, a condition not found in the normal species, where well formed pollen is the rule.

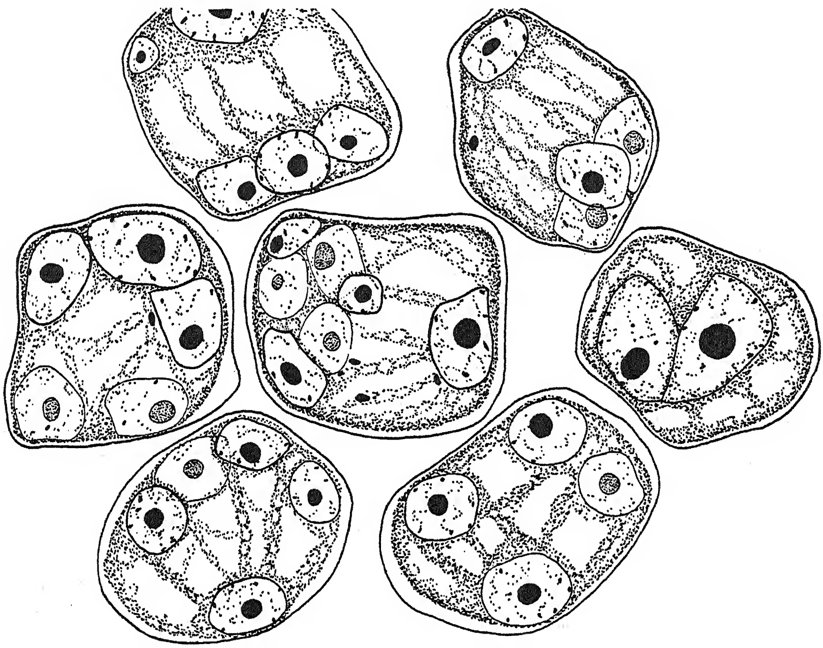

FIG. 2.—*P. anserina, gigas* form: polycary resulting from irregular chromosome distribution during meiosis; $\times$approximately 1200.

Discussion

WULFF (25) made an examination of the pollen of various species of *Potentilla* and found far reaching sterility. After considering the causes of sterility in other cases, namely hybridity, mutation, and environmental factors, he was unable to determine which of these operated to produce *Potentilla* sterility. He believed, inasmuch as percentages varied with geographic location, that sterility was somewhat due to the influence of the outer environment. At the same

time he did not exclude other causal factors, and suggested that hybridity probably occurred in some species; and further, "diese Pollensterilität ist derjenigen bei den der *Potentilla* verwandten Gattungen *Rubus*, *Rosa* und *Alchimilla* analog." Since the time of this allusion, the cytological investigations of LONGLEY (17) on *Rubus*, and of TÄCKHOLM (20) and BLACKBURN and HARRISON (2) on *Rosa*, have shown the connection between divisions and sterility for the first two genera. WULFF found the pollen sterility of *P. anserina* var. *vulgaris* Hayne to range as high as 34.13 per cent. In the *gigas* *P. anserina* there is a still greater amount, and the percentage is often as high as 100.

ROSENBERG's treatment of *Drosera obovata*, *D. longifolia*×*rotundifolia* (19), is a clear-cut case where the irregularities of division are caused by univalent or unpaired chromosomes. Those left outside the daughter nuclei form micronuclei, and in the second division usually develop small spindles. Disorganization appears clearly a little later, when the pollen grains have separated. The protoplasm of the pollen mother cells of this hybrid is "in weniger reichlicher Menge."

As early as 1900, GUYER (10) found that some pigeon crosses were fertile, some crosses non-fertile, and he summed up the features of hybridity as: "(1) abnormalities in meiosis; (2) abnormalities in the structure of the spermatozoa; (3) degeneration of the germinal cells." GUYER believed the conflicting tendencies of parent plasms frequently render the formation of bivalents impossible, and suggested that both cytoplasm and chromatin were involved in the physical basis of heredity.

FEDERLEY (6) studied *Pygaera* species and hybrids, and showed that normal reduction occurs in pure species; in the primary hybrids, on the contrary, there is no conjugation of the "artfremden" chromosomes, and the diploid number is the sum of the haploid numbers of the parents. Abnormalities occur in both divisions but more especially in the second, and, as a result, spermatids with double nuclei often occur. These *Pygaera* crosses are rarely fertile, and the sterility is explained on cytological grounds.

WODSEDALEK's research (24) concerning division and resultant sterility in the mule provides an emphatic case where complete sterility results from the union of gametes, whose chromatin proved

both quantitatively and qualitatively dissimilar. The more recent discoveries of JEFFREY and HICKS (13, 14) disclose meiotic anomalies for *Drosophila melanogaster* quite akin to those described and figured by WODSEDALEK. The elimination of chromatin and the abnormal method of meiosis are considered in this case indicative of heterozygous constitution.

Still more numerous cases of hybridity, both naturally and artificially produced, have been investigated in plants. TISCHLER in 1908 (21) described the cytology of *Potentilla tabernaemontani*×*P. rubens*, as well as that of the parents involved. In the hybrid, the nuclear figures in the dividing pollen mother cells, despite the presence of 16 definite pairs of chromosomes at diakinesis, show that the daughter nuclei receive unlike masses of chromatin, and as many as 20 units have been noted in one nucleus, along with a correspondingly smaller number in the other nucleus. This feature of division, coupled with the deficient amount of cytoplasm, will be recognized to be much like the condition in the sterile *P. anserina*. TISCHLER notes also that frequently in the homotypic only one dyad divides and the other remains undivided. Further irregularities very similar to those in the hybrid are found in one of the parents, for *P. tabernaemontani* shows often an "überzähliger Nucleus" and "versprengte Chromosomen." These are followed in the parent as well as in the hybrid by the production of sterile pollen. Thus there is little difference noted between the parent and the hybrid: "damit dürfte beweisen sein, dass irgend ein prinzipieller Unterschied sich zwischen der Taubheit das Hybriden und der von *P. tabernaemontani* nicht vorfindet."

FARMER and DIGBY (5) found in *Polypodium schneideri* (=*P. vulgare* var. *elegantissimum*×*P. aureum*) that few spore mother cells pass through meiosis, and those that do are abnormal, have small amounts of cytoplasm, and show chromosome lagging. The form is of hybrid origin and the cytology confirms it. However, one of the parents also showed irregular phenomena, and these investigators therefore felt that irregularities do not necessarily connote hybridism, the conclusion earlier reached by TISCHLER.

Miss LJUNGDAHL has recently published accounts of her studies on spontaneous and artificial hybrids of *Papaver* (15, 16), and has shown varying amounts of union between the homologous chromo-

somes; it was observed that union may at times fail completely. Conclusions regarding *Papaver somniferum* × *P. orientale*, very similar to those of Miss LJUNGDAHL, were made earlier by Miss YASUI (26). She noted that F_1 plants were more vigorous than their parents, and states:

> The irregular behavior of chromosomes in the meiotic division, the union of two nuclei, and dropping of certain chromosomes cause the abnormalities of pollen grains, not only in shape and size, but also in the combinations of the heredity substances. This may contribute to the origin of the variation or new forms in the offspring on the one hand, while this may affect the viability of the male gametes and cause the sterility on the other hand.

Thus for *Papaver* crosses, both Miss YASUI and Miss LJUNGDAHL showed: (1) that there is a greater or lesser amount of pairing; and (2) that the lagging of univalent chromosomes on the spindle gives various results.

With the disclosures of the last twenty years showing that abnormal divisions so frequently accompany the reduction division of hybrid plants and animals, these phenomena have come to be regarded as criteria of pure as contrasted with hybrid-originating species. Many "species" have been shown to possess such characteristics as are associated with hybrids.

EKSTRAND'S brief paper on *Plantago major* (4) gives cytological figures which showed irregularities from diakinesis onward, where the chromosomes became shared unequally, and often one or more chromosomes were left outside and formed dwarf nuclei. Such species as *P. psyllium* and *P. depressa* were regular.

HOLMGREN (12) gives figures furnished by the peripheral anthers of *Eupatorium glandulosum*, showing much the same sort of loitering at heterotypic telophase as was viewed in the aberrant *P. anserina;* also regular tetrads never occurred in the species. He considered the constitution of *E. glandulosum* very like that of *Polypodium aureum* × *P. vulgare* var. *elegantissimum*, and of FEDERLEY'S *Pygaera* hybrids. For similar reasons he decided that *Erigeron annuus*, as well as *Eupatorium glandulosum*, arose through hybridization.

GOODSPEED (8) found F_1 hybrids of *Nicotiana sylvestris* × *N. tabacum* var. "*mirdato*" to have uniformly defective pollen. Abnormalities in homotypic division were abundant, and occasionally giant

spindles like those in Miss LJUNGDAHL'S *Papaver* hybrids could be seen. Usually, however, no conspicuous number of chromosomes were left outside.

Miss CAMPIN (3) described irregular methods of pollen formation in *Solandra grandiflora*, and says, "during the reduction division, however, and more particularly during the anaphase of the heterotypic division, there are striking deviations from normal behavior." In this species the chromosomes are extruded to the periphery of the cytoplasm, where they either perish or form accessory nuclei; in most cases they arrive at the poles, but counts of the two nuclei may differ widely: "sometimes 11, 12 or even a higher number of chromosomes will arrive at one pole, while the other has a smaller number, 5, 6 or less." Like the *P. anserina* under consideration, there is a quantitative difference in the chromosomes at the end of the first division.

TISCHLER in 1925 (**22**), after reviewing the more recent investigations, states:

> Seltsam ist es, dass die Abnormitäten, die durch GREGORY (1905) für *Lathyrus odoratus*, durch WHITE (1913) für *Nicotiana* . . . . durch EKSTRAND (1918) für *Plantago major*, durch BEER (1921) für *Geranium ibericum*, etc., beschrieben sind, ganz denen gleichen, die wir bei Bastardiserung kennen lernten.

We may add to this the case of the sterile *P. anserina*, which possesses abnormalities comparable with those reviewed, and which we have come to expect in experimentally produced hybrids.

Conclusions

The irregularities of reduction in the sterile *P. anserina* are probably due to dissimilarity of the parental chromosomes. TISCHLER (**21**) suggested that qualitative agreement of these chromosomes is necessary for subsequent regular chromosome action. As shown by ROSENBERG (**19**), however, lack of quantitative agreement is also responsible for abnormalities. It is suggested that for this *Potentilla* the presence of univalents in addition to bivalents is due to chromosome dissimilarity, either qualitative or quantitative. It is believed that a loose union of the bivalents exists in diakinesis, which allows varying numbers of univalents to appear in metaphases of different cells, and these univalents are responsible for much of the irregu-

larity on the spindle. The abnormalities of division cause the production of various numbers of nuclei at the close of the homotypic. Most of the microspores disintegrate and are factors in the formation of large amounts of sterile pollen.

Notable features are the vegetative luxuriance of the plants, the cytoplasmic poverty of the pollen mother cells, and the appearance of abnormalities of reduction featured by irregular chromosome distribution, which lead to a condition of polycary and eventually of pollen sterility. Finally, this form is usually wholly unable to form fruits.

From a comparison of these with the results of other investigators on plants with known hybrid origin, and on others with suspected heterozygous constitution, one is led to suggest that this infertile *P. anserina* is a product of hybridity.

Summary

1. An aberrant form of *Potentilla anserina* was characterized by gigantism, sterile pollen, and lack of fruit formation.

2. Dividing pollen mother cells of this form reveal irregularities of various sorts.

3. The heterotypic division shows bivalents and univalents, chromosome lagging, irregular chromosome distribution, and the formation of micronuclei.

4. The homotypic division discloses further abnormalities in chromosome distribution, which frequently lead to the development of a condition of polycary.

5. Meiotic irregularities are due to the dissimilarity, either qualitative or quantitative, of the parental chromosomes.

6. Sterile pollen results from the irregular meiosis.

7. This form of *P. anserina* is considered, on the basis of such features, to be a hybrid.

This investigation has been carried on under the supervision of Professor E. C. Jeffrey, and I wish here to express my sincere appreciation of his interest and assistance.

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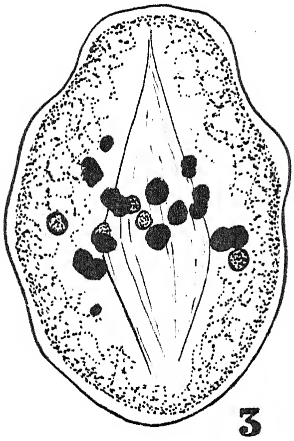
EXPLANATION OF PLATE X

P. anserina L., *gigas* form; pollen mother cells during meiosis; ×approximately 2000.

FIG. 3.—Metaphase, heterotypic.
FIG. 4.—Metaphase, heterotypic.
FIG. 5.—Interkinesis.
FIG. 6.—Metaphase, homotypic.
FIG. 7.—Anaphase, homotypic.
FIG. 8.—Abnormal cell showing three large nuclei formed during meiosis.



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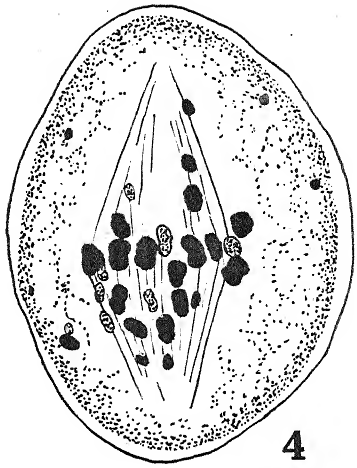

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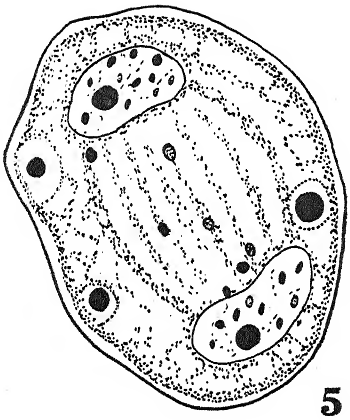

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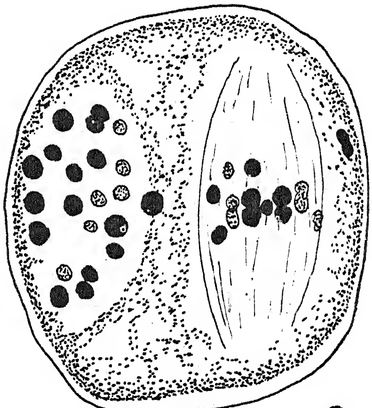

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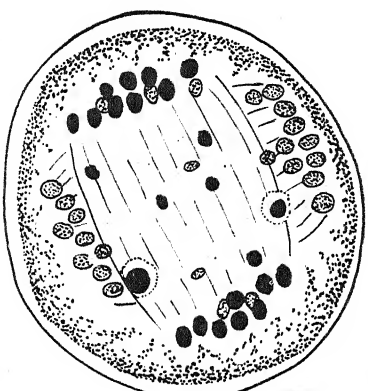

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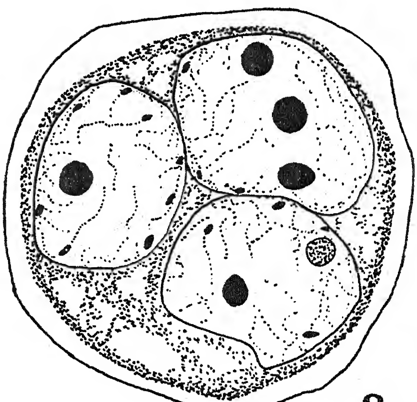

8

ROSCOE on POTENTILLA

CONSTANT RATES OF CONTINUOUS SOLUTION RENEWAL FOR PLANTS IN WATER CULTURES[1]

J. W. Shive and A. L. Stahl

(WITH ONE FIGURE)

In studying the nutrition of plants experimentally, it is very desirable that the initial salt proportions of the culture medium should remain fairly constant during contact with the plant roots. The initial composition of a mixed salt solution used for water cultures may be known accurately, but when the quantity is limited, as it necessarily is in experimental studies of this type, the salt and ionic proportions of the solution begin gradual alteration immediately after introducing into it the roots of vigorously growing plants. Ions move from the solution into the roots as well as in the opposite direction, different ions being absorbed and excreted at different rates, causing the solution to become significantly altered from its original composition where limited quantities are employed. In order that the unknown alterations may be regarded as negligible with respect to their influence upon the growth of the plant, and that growth may be correlated with the chemical conditions surrounding the roots, it is obviously necessary not only that a relatively large quantity of the solution be employed for each culture, but also that this quantity be renewed frequently. The ideal method, of course, is continuous renewal of the culture solution at a constant rate for each culture in the experimental series by some method of "drip and drain." If the rate of flow is rapid enough, the composition of the solution will not be altered significantly by contact with the roots of growing plants, and the roots may then be considered as having been grown under a set of known chemical conditions throughout the period of growth of the plant.

The need of continuous solution renewal in experimental studies with water cultures has long been emphasized, and many investi-

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gators have employed methods by which this was accomplished, without particular consideration, however, of the rates of renewal. As long ago as 1865, NOBBE (4) flowed solution into a vessel containing the roots of growing plants, but he made no attempt to control the rate of flow. In recent years the need of continuous renewal of culture solutions has been emphasized by STILES (6), who urges that the chemical conditions surrounding the roots of experimental plants should be known and as constant and invariable as they can be made. CONNER and SEARS (2) have expressed the same desire for continuous renewal of culture solutions. DUGGAR (3), also emphasizing this need, makes the not too optimistic suggestion that the operation of a system by which this desirable condition might be realized would be impracticable in most experimental work.

TRELEASE and FREE (8) have shown that in solution cultures plants improve in vigor and growth rates as the time interval between solution renewals decreases, but they obtained better results with continuous flow of solution than they did with intermittent daily solution renewal. TRELEASE and LIVINGSTON (9) strongly emphasize the need of flowing solutions in experimental work with water cultures, as do also ALLISON and SHIVE (1), who have shown that solution or sand cultures with continuous solution renewal by which one liter of new solution per culture was supplied during each 24-hour interval throughout the growth period, always produced plants which were superior in every respect to those grown in corresponding cultures with intermittent solution renewal.

It is not particularly difficult to devise a simple means of continuously flowing a solution through a culture vessel containing growing plants when the question of a constant rate of flow is not an important one. But when constant rates of flow must be maintained over a considerable period of time, difficulties immediately present themselves. Some of these have been overcome, in a measure, by a method described by TRELEASE and LIVINGSTON (9). This method is very useful when only one solution is employed for a large number of plants, or when the same solution will suffice for each culture of an experimental series. Because, however, of the labor and expense involved in preparing the apparatus, which is

somewhat complicated, the method becomes almost impracticable when solutions differing in composition must be employed for each culture of a large experimental series. For the continuous renewal of solutions with such a series of cultures, by means of which a constant flow through the culture vessels is maintained, a complete system for this purpose must be provided for each individual culture of the series. This system, to be useful and effective, should be inexpensive, simple in construction, easy to operate, and must provide a continuous flow at an approximately constant rate over an extended period of time. The method here described fulfills these requirements. It has been in use in this laboratory for some time, and in all cases has given entire satisfaction.

Fig. 1 shows the main features of the necessary apparatus, which consists essentially of three parts: the constant level reservoir *A*, the capillary conducting siphon *D*, and the culture jar *B*, with the necessary inlet and outlet tubes *E* and *F*. The reservoir consists of an ordinary one or two-quart fruit jar of colorless glass, which in operation is inverted into a low, flat, glass dish (*C*) of appropriate size. A small dessert dish of colorless glass about 10 cm. in diameter and 3 cm. deep, such as was here used, serves very well. The reservoir is filled with the culture solution, and while still in the upright position, the glass dish is inverted over the opening of the jar and firmly held in position while the jar is being inverted.

The siphon *D* consists of a capillary glass tube about 28 cm. long with 0.5 mm. bore. The tube is bent, as indicated in the diagram, to fit over the edge of the glass dish with the short arm of the siphon long enough to extend several centimeters under the opening of the reservoir jar *A*, and resting on the bottom of the dish. To place the siphon in position, the jar is tilted to one side until the edge of the jar is raised just enough to admit the end of the tube, the rim of the jar then resting on the tube. As the jar is tilted air is admitted, and the solution will flow into the dish until the opening into the jar is sealed. When the siphon is in operation, the solution level in the dish gradually falls until the seal is broken and air is again admitted into the reservoir, when the solution will flow into the dish again to seal the opening. The fluctuation of the solution level in the dish never exceeds 1 cm., however, which is not sufficient to cause any

appreciable change in the rate of flow through the siphon, so that it may be considered as operating from a constant level reservoir. The

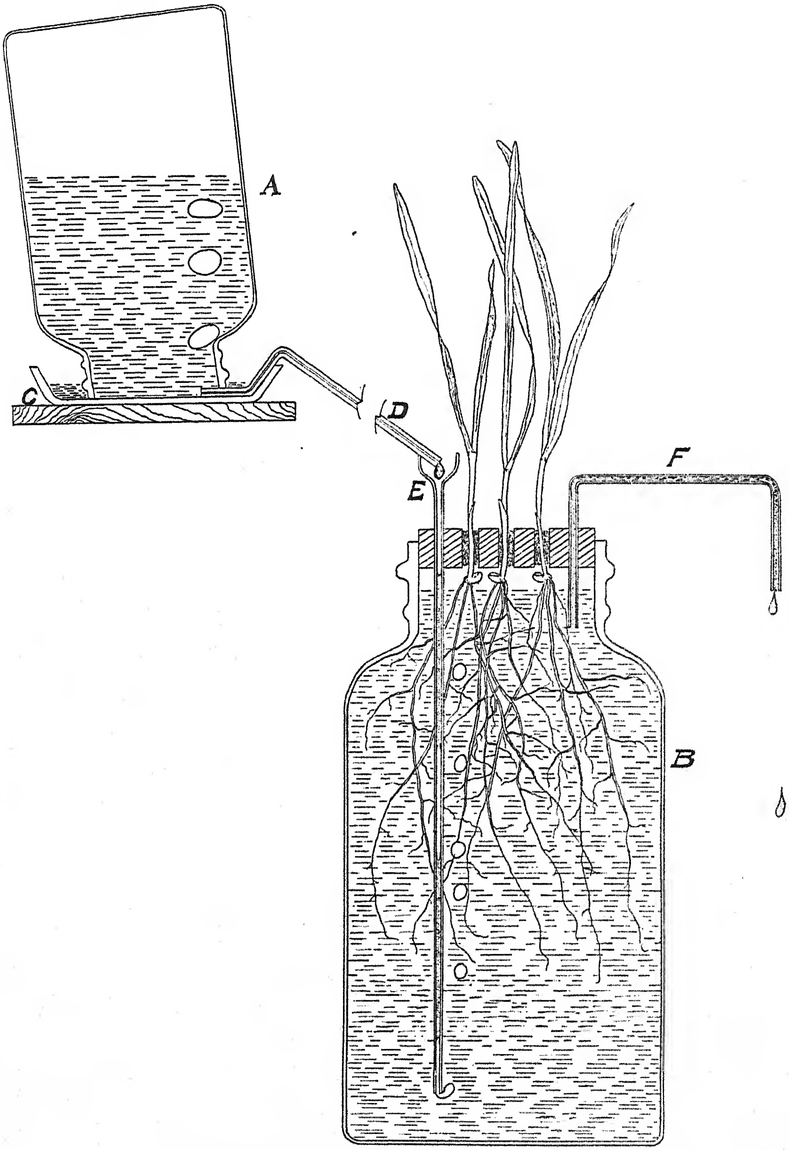

FIG. 1

rate of flow through the siphon is determined, in part, by the length of the capillary resistance, but may be regulated to any rate desired by adjusting the outlet of the tube to the proper distance below the solution level in the dish. By a few trial adjustments, carefully timing the delivery from the tube of several cubic centimeters of solution, the desired rate of flow may readily be obtained. The rate of flow through the capillary tubes is quite uniform when operating from a constant level reservoir. The tubes do not readily clog, as do rubber ones upon which metal clamps are set, and only an occasional cleaning is necessary.

The culture vessel consists of a two-quart fruit jar of colorless glass fitted with a paraffined cork stopper in which the plants are mounted as described by TOTTINGHAM (7). The stopper serves also as a support for the inlet and outlet tubes. The inlet tube *E*, having a bore of about 1.5–2 mm., and with a small funnel at the top, extends almost to the bottom of the culture jar. The funnel on the inlet tube may be prepared by blowing a small bulb about 2 cm. in diameter on one end of the tube. The upper portion of the bulb may then be heated and blown out until exploded, after which the edges may be melted down in a small blast or Bunsen flame to form a rounded funnel. The outlet tube *F*, about 18 cm. long, having a bore of about 2–3 mm., is bent into the form of a siphon, as indicated in the diagram. The arm of the siphon extending through the cork stopper is about 1.5 cm. longer than the other arm. When the culture jar is filled to the proper height, this arm of the siphon extends just below the surface of the solution, which lies in a horizontal plane passing through the other arm just above the outlet. When the system is in operation, the solution surface in the culture jar may be maintained automatically at any level desired by raising or lowering the siphon.

To install the apparatus, a suitable support must be provided to hold the solution reservoir and the glass dish 5–10 cm. above the culture jar, the exact height to be determined by the rate of flow desired. The culture jar, filled with the proper solution and with the plants and tubes supported as indicated in the diagram, is then placed in position so that the end of the siphon will rest on the edge of the funnel at *E*. If the tube is adjusted so that the drip from the

capillary will fall directly into the opening, air will be entrained by the solution and carried to the bottom of the culture jar, thus setting up an aerating system. This desirable feature, however, will not operate if the internal diameter of the tube is too large. A tube with a bore of 1.5–2 mm. is quite satisfactory. The siphon *F* is now started by applying suction, after which it will automatically take care of itself. It is important, however, that the bore of this siphon is not greater than about 3 mm., otherwise the surface tension may not hold the solution in the tube and prevent it from flowing back into the culture jar, if, for any reason, the flow through the capillary *D* should cease. A waste jar will catch the discard solution from the siphon.

To exclude light from the roots of the plants, the culture jar is covered with a cardboard shell in the manner described by SHIVE (5). To prevent undue exposure of the solution surface in the dish, and also to exclude light from the culture solution in the reservoir jar, these parts are also covered when the system is in operation. Waxed paper cans of the proper size, such as are used for ice cream containers, are ideal for this purpose. These cans, with a small rectangular slit cut in the top of each to fit over the siphon tube when the can is inverted over the reservoir jar and dish, afford all the protection that is necessary.

After being installed, the system is self-operating and requires only that the reservoir jar be refilled at the proper intervals and the siphons started after each refilling. The use of this system obviates the necessity of frequently removing the roots of the plants from the culture jar during the experimental period, which in the practice of intermittent solution renewal always subjects the roots to the danger of mechanical injury and infection.

The apparatus is simple in construction, compact, easy of operation, quite inexpensive, and when once installed, a series of cultures each operated under this system requires no more attention and involves no more labor than does a series comprising an equal number of cultures in the practice of intermittent solution renewal. A complete series of 36 cultures, each with its system of continuous solution renewal as here described, has been conducted on a rotating table (5) 5 feet in diameter.

With slight modification, the constant drip apparatus may be used with sand cultures where the solution is permitted to fall upon the surface of the sand in percolators such as were employed by ALLISON and SHIVE (1). As thus far used, the delivery tubes have been calibrated to supply each culture, comprising three plants, with 1 liter of new solution during each 24-hour interval throughout the experimental period.

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CHLORIDE AND SULPHATE ABSORPTION FROM CULTURE SOLUTIONS BY EGYPTIAN AND UPLAND COTTON SEEDLINGS[1]

A. R. C. HAAS

The physico-chemical properties of the leaf tissue fluids have been found to differentiate the Egyptian and Upland types of cotton, in addition to the external botanical characters. An extended series of determinations by HARRIS and his associates (1–5), upon the tissue fluids of the two types of cotton, have yielded the most interesting conclusion that they are differentiated with respect to the osmotic concentration, specific electrical conductivity, hydrogen-ion concentration, chloride and sulphate content. The tissue fluids of the Egyptian types had a higher osmotic concentration, specific electrical conductivity, and chloride content, but a lower sulphate content than those of the Upland types. The hydrogen-ion concentration of F_1 hybrids was intermediate between those of the two parental forms, being higher than that of the Egyptian but lower than that of the Upland parent. The plants were grown under irrigation, and recognition was made of the influence of substratum heterogeneity, which is a universal characteristic of experimental fields (6).

It seemed of interest to study the absorption of chloride and sulphate from culture solutions by Egyptian and Upland types of cotton seedlings, in order to ascertain whether or not the Egyptian type absorbs larger quantities of chloride and the Upland types larger quantities of sulphate. In culture solutions it was possible to maintain a given concentration of the chloride or sulphate ion in the nutrient medium, quite in contrast with the known fluctuations throughout a given soil mass under irrigation (7).

The cultures consisted of one-liter Pyrex beakers with heavily tinned, perforated lids to support the seedlings. The seeds employed were secured from W. B. CAMP, Agronomist in charge of the United States Cotton Field Station at Shafter, California, and from the Uni-

[1] Paper no. 162, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

versity of Arizona at Tucson. Seeds were germinated in pure silica sand, and the seedlings were transferred to the cultures after being carefully washed with distilled water.

Each culture contained a liter of solution. Sodium chloride of a given concentration was added to unmodified Hoagland's solution (10), but when sodium sulphate was the added salt a modified Hoagland's solution containing nitrate instead of the usual sulphate was employed. No sodium sulphate was added to one series of cultures in which the unmodified solution contained 0.3017 gm. sulphate per liter. Twelve seedlings were inserted through the perforations of each lid, the first leaves resting somewhat on the lid and acting as a support. The roots were protected from light by heavy paper wrappers, and the cultures were grown in the glasshouse under controlled conditions. From time to time distilled water containing iron tartrate was added to each culture. Series of cultures were grown at different times of the year.

After making considerable growth, the seedlings were washed with distilled water and the solutions made up to volume. The absorption of chloride or sulphate was indicated by the difference in the weights of chloride or sulphate as determined by the gravimetric method.

Table I gives the percentage of the original concentration of chloride or sulphate absorbed, together with data regarding the growth obtained in several of the cultures. The chloride cultures of May 19, 1926, showed greatest absorption by the Pima or Egyptian type, with a falling off in the absorption at the higher concentration.

In the June chloride cultures the Pima seedlings absorbed the most chloride from the more concentrated solution, but their absorption from the weaker solution was less than that of Acala or Lone Star seedlings. The November chloride cultures showed slightly less absorption by the Pima cultures than by the Mebane or Acala cultures. At the highest concentration of chloride used, the Egyptian type of seedlings showed a greater growth and absorption than the Upland type. At the lower concentrations the Egyptian type did not consistently absorb larger amounts than the Upland type. A larger absorption may result from greater growth of the seedlings, but absorption, growth, and transpiration may not necessarily be

TABLE I

ABSORPTION AND GROWTH OF COTTON SEEDLINGS FROM SOLUTIONS CONTAINING CHLORIDES OR SULPHATES

GROWTH PERIOD	ORIGINAL CONCENTRATION	PERCENTAGE ABSORBED				PLANT GROWTH							
						Pima		Mebane		Acala		Lone Star	
	Gm. per liter	Pima	Mebane	Acala	Lone Star	Fresh wt. of 12 plants (gm.)	Total length average plant (cm.)	Fresh wt. of 12 plants (gm.)	Total length average plant (cm.)	Fresh wt. of 12 plants (gm.)	Total length average plant (cm.)	Fresh wt. of 12 plants (gm.)	Total length average plant (cm.)
						Chloride							
May 19 to June 9, 1926..	0.2610	34.1	19.9	21.5	......	......	......	......	......	......	......	......	......
May 19 to June 9, 1926..	0.5940	17.5	{11.3, 12.5}	12.6	......	......	......	......	......	......	......	......	......
June 9 to July 16, 1926..	0.2855	39.4	33.2	41.3	43.3	48.5	45.0	54.8	37.5	57.5	40.0	52.0	37.5
June 9 to July 16, 1926..	0.5879	43.5	17.6	28.1	15.9	68.5	52.5	36.0	32.5	45.0	31.3	29.5	27.5
November 22, 1926, to January 18, 1927......	0.3080	48.0	53.3	55.4	39.8	58.0	45.0	70.0	30.0	70.0	32.5	49.0	27.5
November 23, 1926, to January 18, 1927......	0.3080	40.3	44.8	43.1	36.5	52.0	40.0	53.0	28.8	54.0	27.5	49.0	27.5
						Sulphate							
May 19 to June 9, 1926..	0.4960	22.2	15.5	13.7	10.7	......	......	......	......	......	......	......	......
May 19 to June 9, 1926..	0.9620	16.0	11.2	11.7	7.8	......	......	......	......	......	......	......	......
June 9 to July 16, 1926..	0.3017	71.3	41.9	66.7	50.9	60.0	55.0	53.0	43.8	58.0	42.5	......	......
June 9 to July 16, 1926..	0.4972	......	31.5	32.6	24.0	......	......	43.0	37.5	52.0	37.5	29.0	33.8
June 9 to July 16, 1926..	0.8903	22.0	7.4	14.9	......	65.0	50.0	36.0	32.5	52.0	40.0	......	......

in any definite relationship to one another (8). The duration of the growth period varied, so that it is not possible to note an effect of the season upon absorption (9).

In the sulphate series the Egyptian type of seedlings absorbed more of the sulphate than did the Upland type during the same growth period. The increased absorption here may be found to be related to the growth of the seedlings. The results suggest that the absorption of chloride and sulphate by the roots of plants may not bear the same relationship to one another in the different types of cotton seedlings as do the chloride and sulphate content of leaf tissue fluids. The absorption of ions by plants, as a basis for distinctions between different forms of cotton seedlings, requires considerable further study under controlled conditions.

CITRUS EXPERIMENT STATION
RIVERSIDE, CALIF.

[*Accepted for publication April 15, 1927*]

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BRIEFER ARTICLES

AN ABNORMAL INFLORESCENCE OF SYMPLOCARPUS FOETIDUS

(WITH ONE FIGURE)

One of the most interesting of swamp inhabiting plants is *Symplocarpus foetidus*, and therefore the observation of any novel characteristic in this species is sure to command attention. In April 1926, SAMUEL W. SMITH of Rootstown, Ohio, sent to the writer an inflorescence of *Symplocarpus* in which the spadix was enveloped by two spathes instead of one. In the ordinary development of all spadiceous plants, the inflorescence is provided with a single bract, and therefore this instance of departure from the normal behavior seemed worth recording.

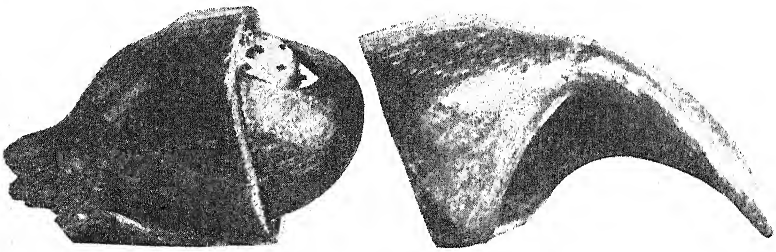

FIG. 1

Fig. 1 shows the orientation of the two spathes. SHULL[1] has shown that *Symplocarpus* in its early life passes through a period of monopodial development, which is succeeded by a sympodial stage. During the monopodial stage the leaves are all rolled in the same direction, but in the sympodial stage the direction of rolling is reversed for each successive leaf. It will be observed that the spathes in the illustration have this same reversed orientation. SHULL has also pointed out that the monopodial stage ends when the terminal bud gives rise to the first spathe. In the sympodial stage the succession shoot bears two leaves, and the upper axillary bud always becomes the new terminal. Concerning this freak

[1] SHULL, J. MARION, *Spathyema foetida*. BOT. GAZ. 79:45–59. 1925.

specimen the question now arises as to the origin of the additional spathe. Does it represent the interpolation of a new primordial region, or rather the modification of a subtending leaf primordium? It would be very difficult to answer this question from the available evidence, but there is some indication that we have here the development of a second spathe primordium in the axillary bud, with the same orientation as that of the leaves.

The writer believes this to be the first *Symplocarpus* inflorescence of this kind ever recorded, for rather extensive inquiry has failed to reveal any reported observations of a similar development.—P. D. STRAUSBAUGH, *West Virginia University, Morgantown, W. Va.*

[*Accepted for publication March 4, 1927*]

CURRENT LITERATURE

BOOK REVIEWS

Plant autographs and their revelations

This little book is a popular presentation of BOSE'S[1] life-long investigations of plant response. The tenor of the presentation is forecast in the preface, where the author remarks concerning the plant that, "in order to reveal the intricate mechanism of its life, it is necessary to gain access to the smallest unit of life, the 'life atom,' and record its throbbing pulsation."

There are twenty-seven chapters and an appendix, which tell the story of sleeping, waking, irritable, nervous, and weeping plants. He recounts the use of his resonant recorder, sleep recorder, automatic recorder, high magnification crescograph, electric probe, electric phytograph, optical sphygmograph, etc., in measuring the otherwise invisible responses of plants under environmental and artificial stimulation. One must admire the genius of BOSE in the invention of delicate recorders of infinitesimal responses. Moreover, the facts of these responses are a challenge to all biologists who are interested in the properties of protoplasm. Everything is in need of explanation and interpretation, for no sane biologist will accept BOSE'S interpretation unless compelled to by critical and penetrating reinvestigation of the phenomena. Some of the former books, presenting more technically the results of his experiments and his reasoning from the observations, are unconvincing. The transpiration stream rise of sap by virtue of pulsating phloem vessels is a theory entirely untenable in the reviewer's opinion. Experiments in this laboratory using BOSE'S bubbler method have failed entirely to confirm his story of sap rise as told in his book dealing with transpiration. We have not been able to find any convincing evidence for a vitalistic interpretation of sap rise. On the other hand, there is no question that many of BOSE'S observations are correct; and correct interpretation of the phenomena, divested of all mysticism, is much needed.

Similarly, the chapters dealing with the so-called nervous phenomena in plants need reinterpretation, as there is no reason for drawing parallels between general protoplasmic conductivity and nerve action of animals. The unfortunate thing about such a book is that it is more enticing to lay readers than a truly scientific account of these same phenomena. If it should make no more impression upon these readers than BOSE'S scientific works have made upon scientists, little harm would be done; but it is more likely that much effort

[1] BOSE, J. C., Plant autographs and their revelations. 8vo. pp. xviii+240. New York: Macmillan Co. 1927.

will be required in attempts to eradicate misconceptions and misunderstandings of plant life from the minds of those whose first contacts with biology are made through such popular and inaccurate representations of life phenomena. As a contribution to the imaginative interpretation of plant life, it should be ranked high.—C. A. SHULL.

Myths and legends of flowers

For those interested in the mysterious, in fables and in legends, a recent volume[2] will furnish many hours of entertainment and amusement. Tales of all sorts connected with flowers and plants are told, many of them borrowed from classical mythology, others from the folklore of various lands. The plants discussed are as various as the sources of the tales. They range from the common wayside weeds such as the thistle and mustard, through the flowers of the garden and the forest such as the lily, the rose, and the violet, to the trees of the wood such as the oak and the cedar, to exotics like orchids and passion flower, and to the less familiar tropical trees like the sal (*Shorea robusta*) and the palms.

Perhaps it is too much to expect scientific accuracy in such a volume, but it does seem that scientific names should be correctly capitalized, and that the real identity of plants designated by common names only should be revealed.

Most of the tales are told in a pleasing manner, so that they may serve to brighten a quiet hour.—G. D. FULLER.

Root development

A new volume on root development of vegetable crops[3] has just appeared from the press, and it is in every way a companion text to WEAVER's *Root development of field crops*, which was so well received last year. The introductory chapter deals in a somewhat general way with questions of plant physiology, ecology, and agricultural practices, pointing out the interrelations between plant and soil. Direct measurements are recorded and applications are made (Chapters II–XXXIV) to forty or more different vegetable plants. Stress generally is placed upon early growth, midsummer growth, mature plants, root habits in relation to cultural practice, soils and fertilizers, and transplanting.

The average reader is sure to be surprised at the extent of the unseen portion of vegetable plants. Without these measurements, who would believe that a horse-radish would send roots 15 feet deep, or that an 8 weeks old sweet-corn plant covers 7 feet with shallow lateral roots? Ninety-four diagrams and photographs are used to show extent and types of roots.

While some data of other publications are included, the book as a whole

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[2] SKINNER, M., Myths and legends of flowers, trees, fruits and plants. Philadelphia and London: J. B. Lippincott and Co. 5th impression. pp. ix+302. *pls. 17.* 1925. $3.00.

[3] WEAVER, J. E., and BRUNNER, W. E., Root development of vegetable crops. 8vo. pp. xiii+351. New York: McGraw-Hill Book Co. 1927.

represents the findings of the authors over several years of research, and since the book deals with problems of so much importance, it will be welcomed by investigators, students, and practical horticulturists.—P. W. ZIMMERMAN.

Check list of forest trees

One of the latest of many valuable contributions by SUDWORTH[4] is a check list of the forest trees of the United States. This volume contains far more than a complete list of names, useful as that would be. To a careful synonymy is added a list of the common names by which the particular trees are known, and also a list of the varieties distinguished in cultivation. In a group of plants like trees, when many an author is a law unto himself in matters of nomenclature, such a carefully annotated treatment of synonyms becomes of great importance. Other topics discussed include the range of each species, trade names for wood, names of hybrid and naturalized trees, and standard names for lumber recommended by the United States Forest Service.

Of the 1177 different tree species recognized, 182 are regarded as of special interest economically. The usefulness of this check list will be all the greater since it may be procured from the Superintendent of Documents, Washington, D.C., for the small price of 40 cents per copy.—G. D. FULLER.

NOTES FOR STUDENTS

Taxonomic notes.—PITTIER[5] has published an account of the Central American representatives of the Lecythidaceae, the brazil nut family. It is stated that "the group has been little studied, since they are mostly large trees, growing in wet virgin forests difficult of access, and it is hard to procure specimens of them." PITTIER describes 5 genera and 19 species, 11 of which are new.

RUSBY[6] has described 10 new species of *Munnozia* (Compositae), a genus which has usually been presented as a section of *Liabum*. The plants are usually erect shrubs, growing at high altitudes in the South American Andes.

In continuation of his presentation of the Fabaceae, RYDBERG[7] describes 31 species of *Hamosa*, additional to the 21 species described in the preceding paper, 8 of which are new. This genus is a segregate from *Astragalus*.

ROSE and STANDLEY[8] have published the species of *Hydrocotyle* from Central

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[4] SUDWORTH, G. B., Check list of the forest trees of the United States, their names and ranges. U.S. Dept. Agric. Misc. Circ. 92. pp. 295. 1927.

[5] PITTIER, H., The Lecythidaceae of Central America. Contrib. U. S. Nat. Herb. 26:1–14. 1927.

[6] RUSBY, H. H., Additions to the genus *Munnozia* R. & P. Bull. Torr. Bot. Club 54:311–320. 1927.

[7] RYDBERG, AXEL. Notes on Fabaceae. IX. Bull. Torr. Bot. Club 54:321–336. 1927.

[8] ROSE, J. N., and STANDLEY, PAUL C., The Central American species of *Hydrocotyle*. Jour. Wash. Acad. Sci. 17:194–199. 1927.

America, based on the abundant material secured by recent explorations. They recognize 8 species, 4 of which are described as new. Two of these new species are remarkably localized, being known only from a single limited locality.

HITCHCOCK[9] has published an account of all the grasses known from Ecuador, Peru, and Bolivia. In 1923 he visited those countries to study the grasses of the Central Andes. It was difficult to identify the specimens, because of the widely scattered descriptions and because the grasses of the region have never been brought together for publication. This paper, therefore, is the first attempt to coordinate all the known grasses of the region. As a result, 605 species are presented, 29 of which are new, included in 124 genera. The following 7 genera include almost one-half of the species: *Paspalum* (54 species), *Panicum* (52 species), *Calamagrostis* (43 species), *Eragrostis* (33 species), *Poa* (28 species), *Stipa* (26 species), and *Festuca* (21 species). There are 52 monotypic genera.

COKER and BRAXTON[10] have published the results of their recent studies of water molds from the soil in North Carolina. A number of new species are described, and also two new genera, *Brevilegnia* and *Calyptralegnia*.

COUCH[11] has published the results of his studies of the water molds collected from the soil around Cold Spring Harbor. Among them he describes two new species of *Brevilegnia*, a new genus recently described from material obtained in North Carolina.

HUTCHINSON and DALZIEL[12] have begun the publication of descriptions of tropical African plants. The first number includes descriptions of 18 new species in 4 families, as follows: Anonaceae 8 species, Lauraceae 1 species, Ranunculaceae 2 species, Menispermaceae 7 species. This work is in connection with the publication of a new flora of West Tropical Africa undertaken by the Royal Botanic Gardens.

In continuation of his work on the flora of Siam, GEDDES[13] has published 20 new species of Rubiaceae, 12 of which belong to the genus *Argostemma*.

SANDWITH[14] has published 14 new species from Argentina, obtained from the collection of H. F. COMBER upon a botanical expedition last year into the Andes of that country.

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[9] HITCHCOCK, A. S., The grasses of Ecuador, Peru, and Bolivia. Contrib. U.S. Nat. Herb. 24:291–556. 1927.

[10] COKER, W. C., and BRAXTON, H. H., New water molds from the soil. Jour. Elisha Mitchell Sci. Soc. 42:139–149. 1926.

COKER, W. C., Other water molds from the soil. *loc. cit.* **42**: 207–226. 1927.

[11] COUCH, J. N., Some new water fungi from the soil, with observations on spore formation. Jour. Elisha Mitchell Sci. Soc. 42:227–242. 1927.

[12] HUTCHINSON, J., and DALZIEL, J. M., Tropical African plants. I. Kew Bull. Miscell. Inf. 1927. no. 4. pp. 150–157.

[13] GEDDES, E. T., Contributions to the flora of Siam. Kew Bull. Miscell. Inf. 1927. no. 4. pp. 164–174.

[14] SANDWITH, N. Y., New species from the Andes of Argentina. Kew Bull. Miscell. Inf. 1927. no. 4. pp. 174–188.

BROWN[15] has described a new genus of Apocynaceae from tropical East Africa and Madagascar. The name *Lanugia* refers to the downy or velvety pubescent inner surface of the corolla lobes. It includes 3 species, 2 of which have been transferred from *Mascarenhasia*.

OGURA[16] has published the results of his studies of specimens of fossil stems and petioles obtained from Mesozoic deposits in Japan. Three new genera are described, namely, *Cyathocaulis*, *Cibotiocaulis*, and *Cyathorachis*. The descriptions are very detailed and well illustrated. The author concludes that these genera are related to the Cyatheaceae. The exact systematic position could not be determined because of the lack of fronds.

KILLIP,[17] in preparing a revision of American Passifloraceae, has discovered some new species which he publishes in advance of the publication of the completed revision. Twelve species of *Passiflora* are described, 2 from Mexico, 1 from Cuba, and the remaining 9 from various countries of South America.

Miss KANOUSE[18] has begun the publication of her investigations of certain groups of water molds concerning which there has been very incomplete information. In the present paper 23 species of Blastocladiaceae are presented, the experimental work and the morphological results being fully described. A new order, Leptomitales, is established; also a new genus, *Mindeniella*, 3 new species of *Blastocladia*, and 1 new species of *Allomyces*. Twelve of the species included in this monograph were collected in the vicinity of Ann Arbor, Michigan.

DANDY,[19] in connection with his investigation of Magnoliaceae, has described 4 new genera from Asia, namely, *Alcimandra*, *Pachylarnax*, *Elmerrillia* (including 5 species segregated from other genera), and *Kmeria*. In addition, he publishes 10 new names and combinations. His conclusion is that "much of the confusion which for a long time has existed in this group is due to the various interpretations of the limits of the genera."

TRELEASE[20] has published 13 new species of *Phoradendron*, an exclusively American genus, which have come to his attention since the publication of his monograph. The majority of the species are from Ecuador, Venezuela, and Colombia.

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[15] BROWN, N. E., *Lanugia*, a new genus of rubber-yielding trees. Torreya **27**:51–53. 1927.

[16] OGURA, YUDZURU, On the structure and affinities of some fossil tree ferns from Japan. Jour. Imp. Univ. Tokyo. Section III. Botany. **1**:351–380. *pls. 2–8*. 1927.

[17] KILLIP, E. P., New passion flowers from South America and Mexico. Jour. Wash. Acad. Sci. **17**:423–431. 1927.

[18] KANOUSE, BESSIE B., A monographic study of special groups of the water molds. I. Blastocladiaceae. Amer. Jour. Bot. **14**:287–306. 1927.

[19] DANDY, J. E., The genera of Magnolieae. Kew Bull. no. 7. pp. 257–264. 1927.

[20] TRELEASE, W., Additions to the genus *Phorandendron*. Bull. Torr. Bot. Club **54**:471–477. 1927.

BLAKE[21] has described an endemic group of species of *Aplopappus* occurring in the Andes of northwestern South America. Formerly this group had been regarded by BENTHAM and HOOKER as a section of *Chrysothamnus*, and by GRAY as a section of *Bigelovia*. BLAKE has now transferred it to *Aplopappus*, describing 9 species, 5 of which are new.

STEVENS[22] has described 3 new genera of tropical fungi. *Shropshiria* is from Panama, and its relationship is said to be problematic. For the present it should probably be included among the Fungi Imperfecti, but it shows no relationship to the 3 orders of that assemblage. *Clypeodiplodina* is from Ecuador, and probably belongs to the Pycnidiaceae. *Chaetothyriopsis* is from Panama. It is an invisible fungus except with the aid of a compound microscope.

PAINE[23] has been investigating the fungi of virgin soils, since the greater amount of work has been done with cultivated soils in the interest of agriculture. The fungi were isolated from pasture land and timber land near Iowa City, Iowa. The investigation resulted in the discovery of 30 species, 5 of which are described as new. "There was a marked diminution in the frequency of occurrence of fungi with increase in depth beyond the first 3 inches, but the lower depths yield their proportion of the new species, 4 of the 5 new forms found being isolated from 6 to 12 inches below the surface."

GARDNER[24] has published descriptions of 11 new species of *Gelidium* discovered along the coast of California. These seaweeds grow chiefly in the sublittoral belt, although some of them occur in deeper water. The descriptions are accompanied by 19 exceptionally good plates.

TRELEASE[25] has published a revision of the Piperaceae known to occur in Panama. The family is remarkably well represented in that region. Doubtless many more species will be found, since no part of Panama has been explored for such material except the region of the Canal Zone. The present revision includes 139 species, *Piper* containing 92, and *Peperomia* 44. A new genus, *Sarcorhachis*, is described, based upon a species formerly included in *Piper;* 42 new species are described in *Piper*, and 19 in *Peperomia*.—J. M. C.

Alpine vegetation related to alpine soils.—The advantages of associating with an ecologist a collaborator trained in soil science, is seen in a recent

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[21] BLAKE, S. F., The section Diplostephioides of *Aplopappus*. Amer. Jour. Bot. **14**:107–115. 1927.

[22] STEVENS, F. L., New tropical fungi. Mycologia **19**:231–238. 1927.

[23] PAINE, F. S., Studies of the fungous flora of virgin soils. Mycologia **19**:248–367. 1927.

[24] GARDNER, N. L., New species of *Gelidium* on the Pacific coast of North America. Univ. Calif. Publ. Bot. **13**:273–318. 1927.

[25] TRELEASE, W., The Piperaceae of Panama. Contrib. U.S. Nat. Herb. **26**:15–50. 1927.

investigation of the vegetation of Central Switzerland by BRAUN-BLANQUET,[26] who has been assisted in his interpretation of soil phenomena by HANS JENNY. In the humid conditions of the alpine climate soil development tends to proceed from basic or weakly acid conditions to a strongly acid climax, and this evolution is irreversible. It is demonstrated that correlated with these changes in soil conditions there is a definite plant succession leading to a definite climax vegetation. In many parts of the Alps the climax association is one characterized by *Carex curvula*. This oxalophytic association agrees in its distribution with areas occupied by the strongly acid climax soil, the alpine humus soil. In regions of siliceous rocks these climaxes of soil and vegetation are of common occurrence, but upon substrata of limestone the soil changes are so slow and gradual that the climax stages are only occasionally found upon gentle slopes and flat-topped ridges.

The calcicolous species are usually the pioneers, and represent plants of great constructive value in the development of the association. Presently, however, the acid tolerant and acid demanding species come into competition with species having basic soil preferences, and with the development of the soil climax the former become more abundant. Thus as the plant succession develops, the range of toleration for pH soil values is narrowed and limited to those of rather high acidity.

Among the phases of the subject investigated in a quantitative manner are the great importance of wind-blown material and the ratio between humus production and humus destruction. The development of alpine soils is shown to be from the "braunerde" or "rendzina" stage, through the podsol stage to the climax of the alpine humus soil.—G. D. FULLER.

Desert vegetation illustrated.—The strikingly characteristic plants of the Arabian and Egyptian deserts are brought graphically before us by STOCKER,[27] in a recent issue of the *Vegetationsbilder*. The score of plates maintain the high character of excellence peculiar to this publication. They include several good landscapes, and also habit studies of species of *Zygophyllum, Erodium, Cotula, Capparis, Statice,* and *Zilla*.—G. D. FULLER.

[26] BRAUN-BLANQUET, J. (with JENNY, HANS), Vegetations-entwicklung und Bodenbildung in der alpine Stufe der Zentralalpen (Klimaxgebiet des Caricion curvulae). Denkschr. Schweiz. Naturf. Gesell. **63**:175–349. 1926.

[27] STOCKER, OTTO, Die Agyptesch-Arabische Wuste. Vegetationsbilder, KARSTEN & SCHENCK **17**: heft 5–6. *pls. 25–36*. 1927.

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THE

BOTANICAL GAZETTE

December 1927

ANATOMY OF LEAF OF BANANA, MUSA SAPIENTUM L. VAR. HORT. GROS MICHEL[1]

ALEXANDER F. SKUTCH

(WITH FORTY-FOUR FIGURES)

I. External features

1. DISTRIBUTION OF LEAVES ALONG STEM; PSEUDOSTEM

Until a certain stage in the development of the plant, the entire aerial portion of the banana is made up of leaves alone. To a superficial observer, the banana of this age appears to consist of an erect, unbranched stem surmounted by a crown of enormous leaves, to be, in fact, a "tree," as it is often loosely designated, with a "trunk" 3–4 m. high and 15 cm. or more in diameter. If, however, the "trunk" is cut across at any level above the base, it is found to be a pseudostem composed of the overlapping, close-fitting leaf sheaths alone, and containing no axial member at all. As the plant approaches maturity, the condensed stem at its base begins to elongate and to push upward in the center of the pseudostem, finally emerging at its upper extremity, where it bends downward and produces the pendent inflorescence. The leaf insertions, which previously were in contact on a stem condensed into a bulb, are more and more widely separated along the lengthening axis, and internodes of increasing length appear.

[1] Botanical contribution from the Johns Hopkins University, no. 88. This investigation was made and its results are published with the aid of a grant from the Department of Agricultural Research of the United Fruit Company.

Whether or not the banana has a true aerial stem is a question more of academic than of practical importance. As SCHUMANN (20) observes, the phyllotaxy, whether distichous and alternate or spiral with a smaller divergence, is of more importance in determining the appearance of a genus of the Musaceae than the degree of development of the axis. However, since the theoretical interpretation is apt so to color the mere statement of the actual situation that if it is a mistaken one a false impression will be left in the mind of the reader, it seems desirable to re-examine the facts. Most brief accounts of the structure of the banana plant, particularly the more or less popular ones (4, 18, etc.), place so much stress upon the peculiar pseudostem, contrasting it with the "true" stem or bulb at its base, and with the floral axis which pushes its way upward through the former toward the light, that an erroneous picture of the aerial stem is very naturally formed. One might well expect, after reading these accounts, to find the inflorescence borne on the end of a gigantic, leafless scape, naked except for the bracts just beneath the flowers. Accordingly, the writer was considerably surprised, when he made his first longitudinal section of an entire plant, slicing it up the center with a long cutlass, to find the stem leafy almost to the summit (table I). WITTMACK (22) gives a clear statement of the facts, but the writer was unable to obtain this article until later. The other accounts just referred to contain no actual misstatement of the case, but are at fault rather from lack of completeness, and in particular from false emphasis.

Table I gives the sizes and heights of insertion of all of the leaves present on two fruiting plants. In these plants, respectively 396 and 422 cm. high, the largest leaves were inserted 30–150 cm. above the ground, and the absolutely largest at 69 and 67 cm. respectively, while the stems bore true foliage leaves to about 3 m. above the base. In other words, more than the basal three-quarters of that portion of the stem within the leaf sheaths was leafy. WITTMACK records the heights of insertion of the leaves of a specimen of *M. ensete*, which resembles *M. sapientum* in the distribution of leaves along almost the entire inclosed portion of the stem. The sizes of the leaves are not recorded in his table, but the largest leaf scar, and presumably the largest leaf, occurred at 49 cm. from the ground. On the

other hand, the stem which bears these leaves is totally incapable of supporting them without the aid of the inclosing sheaths. Whether or not the elongated portion of the axis which bears the largest leaves is to be considered a true stem, as well as the condensed basal portion which bears most of the leaves, is a question of definitions. Morphologically it is certainly stem, whatever we may choose to

TABLE I

SIZE AND DISTRIBUTION ALONG STEM OF INTACT LEAVES OF TWO FRUITING PLANTS

Number of leaf	Plant I* Pseudostem = 396 cm. high			Plant II† Pseudostem = 422 cm. high		
	Height of insertion	Length of leaf	Greatest width	Height of insertion	Length of leaf	Greatest width
1	Basal	262 cm.	79 cm.	19 cm.	274cm.	79 cm.
2	Basal	178+‡	86	22	274	86
3	8 cm.	279	84	24	282	Withered
4	9	292	84	28	277	89
5	14	284+‡	89	29	300	89
6	18	239+‡	86	36	310	91
7	25	302	97	43	323	91
8	32	305	94	53	315	91
9	43	330	99	67	328	84
10	69	330	97	98	318	84
11	109	320	99	146	300	84
12	180	305	86	224	272	75
13	277	226	74	318	178	56
14	363	Dead§	Dead§	387	Dead§	Dead§

* 15 sheaths, totally or partially alive, the laminae belonging to which had fallen away, were removed from the outside of the pseudostem.

† 16 sheaths were removed.

‡ End of lamina torn off.

§ "Protecting leaf."

call it in regard to function. The banana may well be likened to any of our familiar perennial herbs of field or garden, which during the first year form a basal rosette of leaves at the crown of the rhizome, and in the succeeding season produce a leafy, aerial, flowering stem; only in the banana, a tropical plant, there is no resting season, and the circumstance that the leaves of the rosette are erected into a pseudostem which conceals and supports the true aerial stem is apt to be confusing. Even the pseudostem is not without representatives among temperate plants. The leek furnishes a good but diminutive example, although here the flowering shoot is lateral and not cen-

tral, as in the banana. The celery plant forms a larger but rather loose pseudostem.[2]

2. Phyllotaxy

The phyllotaxy is not constant throughout the life of the plant, but the angle of separation between successive leaves increases with the age and size of the individual. The fraction expressing the spiral of phyllotaxy was determined both by counting the number of leaves and of turns between two superposed leaves on the pseudostem, and by studying the arrangement of the sheaths of cross-sectioned pseudostems. Consistent results were obtained by the two methods, except in the case of very young suckers, where, probably as a result of a slight torsion of the pseudostem, the leaves often show a divergence very close to 1/3, although the angular separation as determined by the sheaths is nearer 2/5. The actual separation of the sword leaves of a young "ratoon," 50 cm. high or less, is often very slightly over 1/3. Somewhat larger ratoons show very clearly a phyllotaxy of 2/5, both of the blades and of the sheaths. At a somewhat later stage the spiral becomes clearly 3/7, both in the blades and sheaths. The size of the plant when the transition occurs depends largely upon its situation. A ratoon growing up at the base of a large plant, where it is shaded and produces sword leaves for a long time, may retain the 2/5 arrangement until it is over 120 cm. high, while an isolated sucker planted in the open, where it soon forms broad leaves, may show a 3/7 phyllotaxy at less than half that height. The two arrangements may often be seen in the same plant, the divergence between the older (lower) leaves being 2/5, that between the younger (upper) 3/7. Mature plants always have a phyllotaxy of 4/9, which may occur in plants 2 m. high, and is very clearly maintained in plants bearing full bunches of fruit, the presence of the bent-over stem causing no confusing distortion of the spiral.

The angular separation of the leaves of a very young ratoon, then, is slightly more than 120°. At a somewhat later stage, the age and size of the plant depending upon the environment, it becomes 144°

[2] *Veratrum album*, among temperate monocotyledons, provides an excellent example of the pseudostem. See Mrs. Arber's *Monocotyledons*, fig. xxxiii (Cambridge, 1925).

(in the ideal case). Still later it increases to 154°, and in full grown plants and those bearing fruit, the angular divergence is 160°.

WITTMACK gives the phyllotaxy of *M. ensete* as 3/7. GREVE (8) repeats this statement, and adds that it is true for all species of *Musa*. In young seedlings he found the phyllotaxy to be 1/2. SCHUMANN (20) records the 3/7 spiral as general for the Musoideae. In addition to examining numerous examples of the Gros Michel variety on the plantation, the writer had the opportunity to inspect the varieties Apple, China, Honey, Ramkelat, Robusta, and White House, as well as "*Musa kewensis*," in Hope Gardens, Jamaica. Mature fruiting plants of all of these showed a divergence of 4/9, younger plants 3/7 or less, according to their age. A single fruiting plant of the slender-stemmed *M. kewensis* maintained the 3/7 divergence of its leaves to the last.

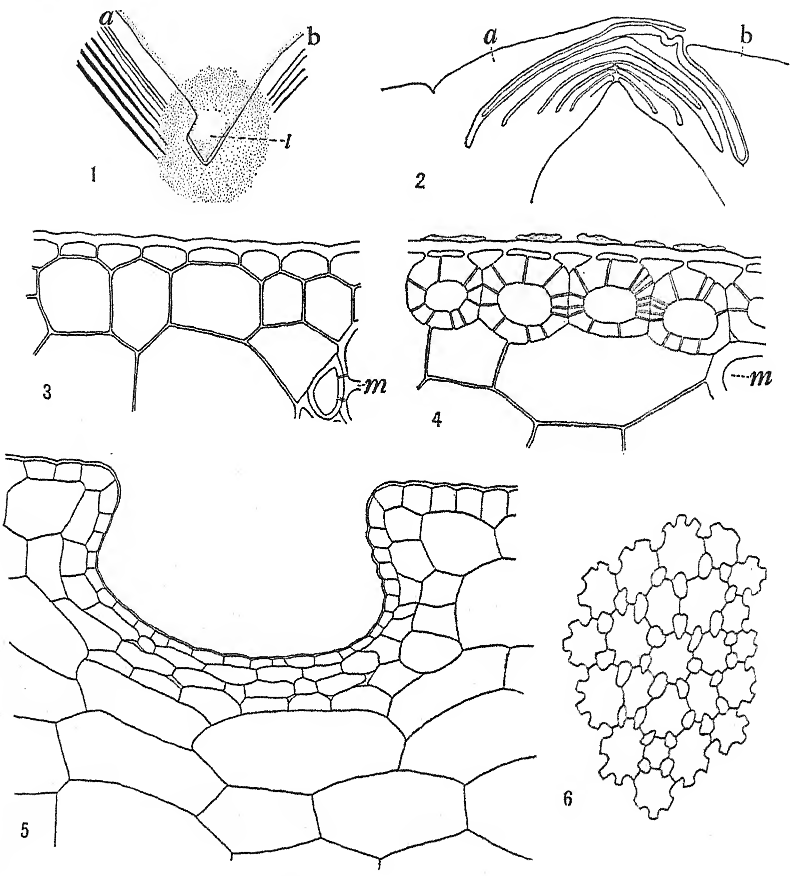
The spiral in which the leaves are arranged is always left-handed; it rises from the right to the left of an observer facing the stem.

At their insertion the leaf sheaths entirely surround the stem, the scar forming a complete circle. The insertion may be somewhat oblique, and in some of the higher leaves is markedly so. Here the vertical distance between the highest and lowest point of the scar amounted in one instance to 8.8 cm. where the stem was only 5 cm. in diameter. There seems to be no rule as to which point is uppermost; any point on the entire circumference of the scar may occupy that position. WITTMACK found that in *M. ensete* the scar does not completely surround the stem, but occupies 59–80 per cent of the circumference. He records even larger obliquities, amounting to 22 cm. The diameter of the stem is always considerably less just above the leaf insertion than just below it.

3. LATERAL BUDS

The lateral buds do not occur in the axils, as stated by SCHUMANN, but are situated opposite them. They regularly appear in the angle of the V formed by the two margins of the sheath as they converge to the point of insertion (fig. 1). The scarious margins overlie the outermost scales of the bud (fig. 2). Buds occur regularly at the insertion of each of the basal leaves of the plant, but not of those situated in the region of elongated internodes, that is, the

aerial stem. The position of the buds suggests that possibly the stem is a sympodium, but the point has not been investigated anatomi-



FIGS. 1–6.—Fig. 1, lateral bud (*L*) in surface view; *A*, *B*, margins of sheath of about 16th leaf from center of pseudostem; ×1. Fig. 2, horizontal section through similar bud; ×10. Fig. 3, outer epidermis and subjacent cells from a sheath near inside of pseudostem; *M*, portion of fibrous strand; ×400. Fig. 4, same but from exposed sheath of same plant; ×400. Fig. 5, pit from inner surface of sheath, in horizontal section; ×150. Fig. 6, stellate cells from transverse septum of sheath; ×36.

cally. In the related *Ravenala madagascariensis* the buds are truly axillary.

4. Leaf parts

Four regions along the length of the leaf are distinguished, but the divisions are suggested by convenience of treatment entirely without regard to morphological considerations, and the transition

Fig. 7.—A "protecting leaf" which hangs over a bunch of immature fruit

from one region to another is gradual and not sharp. These are, from the apex downward: the precursory appendage,[3] the lamina, the petiole, and the sheath. The appendage is a temporary organ, and has already withered when the leaf reaches maturity. The lamina contains a strong midrib which is flanked on either side by a pulvinar band. This is followed by a broad blade, bordered in the

[3] So I translate the *Vorläuferspitze* of German authors.

young leaf by a scarious margin which soon withers away. The petiole is a region of transition from the sheath to the midrib, in the larger leaves reaching 30 cm. or more in length. The sheath may be defined as that portion of the leaf which participates in the formation of the pseudostem.

5. Size and form of lamina

The lamina of the banana, in its pristine state as it emerges from the pseudostem, represents one of the largest unbroken expanses of photosynthetic tissue in the entire plant kingdom. In the largest leaves of the Gros Michel it attains a length of about 4 m. and a maximum breadth of over 1 m. It is exceeded in size notably by the enormous leaves of another member of the Musaceae, *Ravenala madagascariensis*, which are stated to attain 6 m. in length, by the floating leaves of *Victoria regia*, the largest of which reach 4 m. in diameter, and by the blades of certain kelps, such as *Laminaria longicruris*, attaining 6 m. in length and 1 m. in breadth. They are approached and perhaps equaled in area by the leaves of certain species of *Gunnera*. Of course, many tree ferns and palms have leaves of greater dimensions, but these are all compound, and not directly comparable with those of the banana. It is a significant fact, the bearing of which will be evident when we consider the splitting of the lamina by the wind, that the banana and its relatives bear perhaps the largest undivided aerial leaves to be found anywhere. Reliable data on the size of leaves are difficult to obtain.

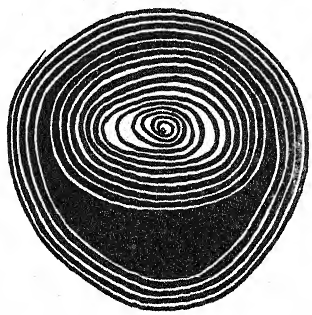


Fig. 8.—Transverse section of leaf as rolled inside pseudostem, showing form of vernation; ×3.

The ovate-oblong leaves of the mature form are the last of a long series, the first of which are strikingly different in both size and shape. The first leaves arising from the subterranean bud, which grows out from the bulb to form the replacement shoot or ratoon, are reduced scale leaves, in which the portion that represents the sheath alone is prominent. In the succeeding leaves the lamina

becomes more and more conspicuous, first in the form of the long narrow "sword leaves" characteristic of the young ratoon, and as the plant grows older, gradually increasing in size and relative

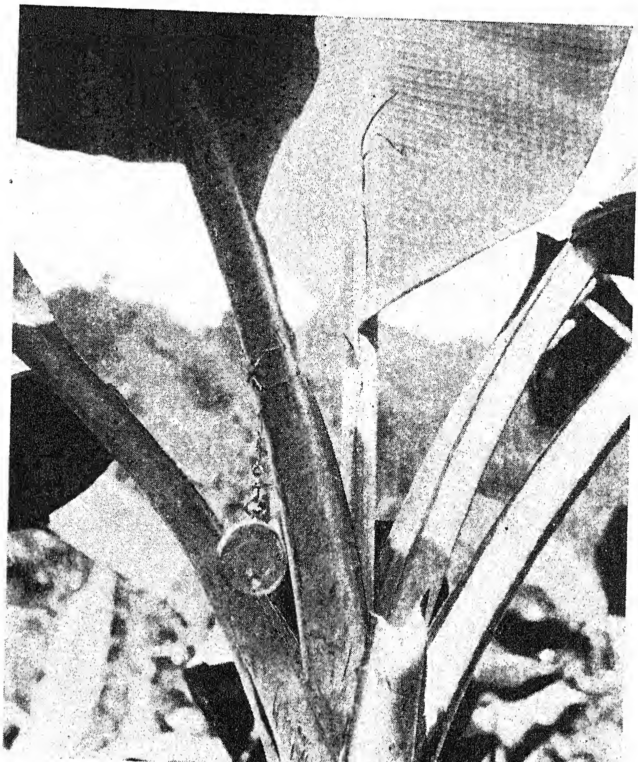


FIG. 9.—Young leaf just emerging from pseudostem; note long precursory appendage.

breadth until the mature proportions are attained.[4] The rapidity of change depends upon environmental conditions, of which light is probably the principal. Isolated suckers form broad leaves much

[4] According to COOK (3), there are two kinds of suckers, which differ in the character of their early leaves. The "broad-leafed" suckers originate from buds situated near or above the surface of the ground, and produce broad leaves from the first; the "sword" suckers arise from more deeply buried buds, and produce the characteristic linear leaves. For many excellent illustrations of leaves of both forms, and of the plant as a whole, see REYNOLDS (19).

earlier than those still attached to the parent plant. The fruiting plant retains about 11–14 intact leaves, together with about 12–16 sheaths which are still in part or entirely succulent and aid in the support of the stem, and perhaps serve as storage organs, although their blades have withered and fallen.

FIG. 10.—Leaf which has emerged from pseudostem to almost full length of lamina, but which has not yet begun to uncoil, at top of figure near middle.

6. "PROTECTING LEAF"

The last of the series of green vegetative leaves differs considerably from those just below it, and is distinguished as the "protecting leaf." In comparison with the other leaves it is very much reduced in length, but about equally broad (fig. 7). The broad flat

petiole retains a sheathlike structure, and clasps the inflorescence axis as it emerges from the top of the pseudostem, preventing more or less completely the penetration of rain water between the innermost sheaths and the stem. It is incapable of supporting the lamina, which bends forward and usually hangs for a time over the inflorescence, affording it temporarily a certain degree of protection from sun and rain. This leaf is inserted very near the top of the pseudostem, in the plants measured 30–40 cm. from its apex. It is short-lived, and droops and withers before the fruit is mature.

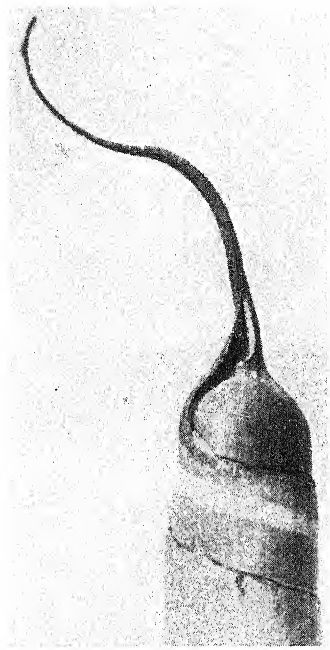


FIG. 11.—Dome which caps lamina just before it begins to uncoil, surmounted by partially withered precursory appendage.

7. VERNATION

In vernation the leaf is convolute. The rolled right[5] half of the lamina, making about 18 turns at its broadest portion, fits neatly into the concavity of the upper side of the midrib, and the whole is surrounded by the 4–5 turns of the left half (fig. 8). Preceded by the precursory appendage, the coiled leaf pushes upward from the center of the sheath of the one next in age (fig. 9), and emerges to practically the full length of the lamina as a straight, stiff, green rod (fig. 10). Then the coils begin to loosen, and the leaf first expands at the apex. The hollow cylinder which is formed just before the leaf spreads out terminates in a dome or cupola which is surmounted by the precursory appendage (fig. 11). The right half of the lamina alone participates in the formation of this dome, while the left half, rolled about the outside of the cylinder, may readily uncoil and spread out. Since the tissues of the dome undergo no

[5] As reckoned by an observer standing at the petiole and looking toward the apex of the leaf in its normal orientation.

further expansion, mechanical considerations make it at once evident that the right half cannot unroll without tearing away the permanently curved portions. The rupture of tissues occurs principally along the veins, and the dome is removed more or less intact. Usually the dome with the appendage remains at the end of the midrib, but sometimes they are torn from the latter and carried to

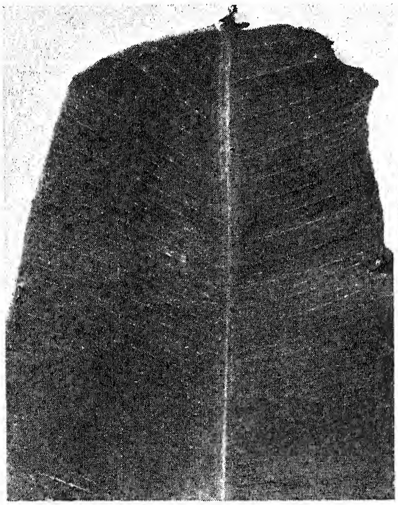


Fig. 12

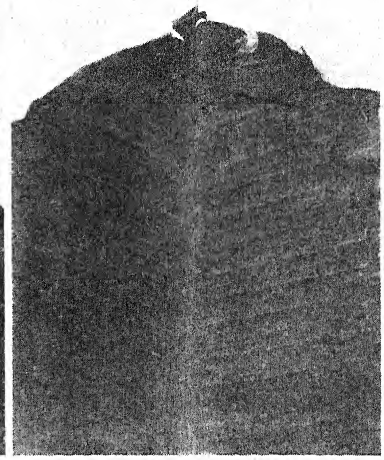


Fig. 13

Figs. 12, 13.—Apices of lamina of two leaves which have just uncoiled, showing torn right end and remains of dome and precursory appendage; in fig. 12 tear is straight and dome intact, in fig. 13 tear is more irregular.

the right side of the leaf. The line of separation is evident throughout the life of the leaf, and is more or less regular, according to whether the tear follows a single vein (fig. 12) or strikes across the lacunae from one vein to another (fig. 13).

II. Anatomy

A. SHEATH

8. Gross structure

The sheaths innermost at any particular stage of growth extend for practically the entire height of the pseudostem. Exception must be made, of course, for the leaves inserted along the late-appearing

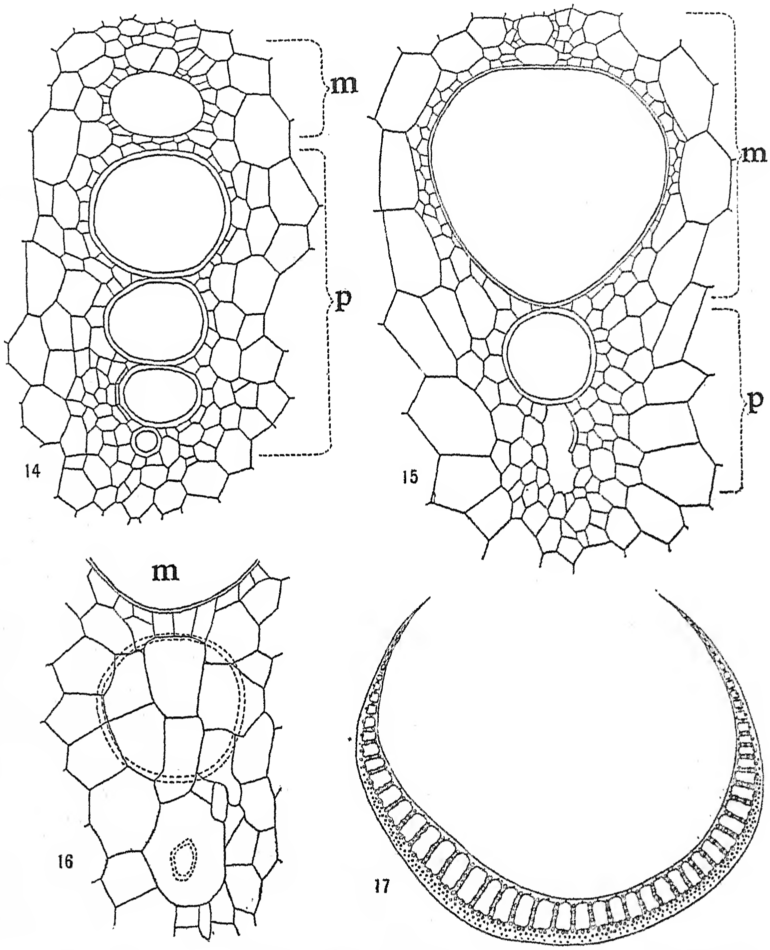
aerial shoot. Although the margins of the young sheath overlap and the sheath forms a hollow cylinder, the older, more exterior sheaths are typically crescentic in cross-section (fig. 17). The thickest point is near the center, whence the cross-section tapers to a thin transparent wing, which contracts at the margin to the thickness of two extremely narrow cells (about 9 μ). At the base the maximum thickness of the sheath is about 14–17 mm., at the top, about 10 mm. At its insertion on the stem the sheath completely surrounds it. It narrows abruptly to about half the initial breadth at a point 30 cm. higher up, whence it contracts gradually to the petiole. At their bases, old sheaths are penetrated while still alive by roots arising from that portion of the stem inclosed by them.

Between a thick outer and a thinner inner wall is situated a series of lacunae which occupy the bulk of the volume of the sheath. A series of radial septa runs the entire length of the sheath and divides the cavity between the walls into longitudinal canals. These are interrupted at frequent intervals by thin horizontal septa, with the result that a series of rectangular air chambers is formed. In the widest portion of large sheaths the lacunae are about 5–8 mm. wide (tangential dimension), 8–11 mm. deep (dimension normal to surface of sheath), and 2–6 mm. from top to bottom. At the base of the pseudostem the inner wall of the sheaths is often strongly corrugated, and the horizontal septa strikingly folded. This condition is reminiscent of the wavy grain of the wood at the base of large trees, and is a result of the same mechanical stresses in tissues of the same function but very different homology.

9. Dermal system

The epidermis is composed of straight-walled cells, rectangular in surface view, with their long axes parallel to the length of the sheath. Beneath the epidermis is a hypodermal layer of cells having their long axes parallel to those of the epidermis in the case of the outer surface, but perpendicular to them in the inner surface. Stomata occur on both the outer and inner surfaces of the sheath throughout its length, as well as on the inclosed portion of the stem even to its base. On the inner surface of all the sheaths and on the stem the stomata are not exposed to the atmosphere, since the

sheaths fit very tightly together. On the outer surface of some sheaths they are after a long period exposed, but here also on many



FIGS. 14–17.—Fig. 14, xylem portion of vascular bundle of sheath of leaf just emerging from pseudostem, section cut at about 5 mm. above insertion of sheath; protoxylem elements (*P*) still intact, metaxylem (*M*) immature; ×160. Fig. 15, bundle from sheath of about same age, section at 24 cm. above insertion of sheath; earliest protoxylem elements disrupted, and large metaxylem element almost mature; ×160. Fig. 16, portion of mature bundle, showing site of occluded protoxylem elements; annular thickening and turn of spiral band, slightly below plane of drawing, indicated by broken lines; ×285. Fig. 17, transverse section of sheath from fruiting plant, position of principal vascular bundles indicated by circles; ×about 0.5.

sheaths they are never brought into contact with the air. The presence of these stomata seems a result of phyletic inertia rather than physiological advantage. The average density of stomata on the outer surface was found to be 11 per $\overline{mm}.^2$ on the inner surface, 7 per $\overline{mm}.^2$ near the base, and 12 per $\overline{mm}.^2$ at the top. The density of stomata on the stem itself is about the same as on the sheaths. The stomata agree in general form with those described later for the lamina, but the cuticular ridges are more pronounced, the front and back cavities larger, and the substomatal chamber narrower and deeper. They are larger than those of the lamina, and those on the inner surface are larger than those of the outer surface, agreeing with the larger stomata on the upper rather than on the lower surface of the lamina. Length of stomata on inner surface is 34–38 μ, outer surface 27–34 μ.

The character of the epidermis does not vary greatly according to whether it is taken from a sheath near the center of the pseudo-stem, or from one which is exposed to the air. A very thin cuticle overlies a thick wall of cellulose (figs. 3, 4). In places a waxy layer is present over the cuticle. The cutinization of the guard cells resembles that to be described for the lamina. The epidermal layers of two sheaths in contact adhere so strongly that in stripping off a sheath it is a common thing to find that the thinner portions at the edge remain attached to the one within, and tear away from that which is removed.

The cells of the hypodermal layer of the outer surface show a profound difference in form and chemical composition, according to whether an outer exposed, or an inner protected sheath is taken (cf. figs. 3 and 4). If an inner sheath is selected, say one which is covered by four or five others, or any nearer the center than this, the hypodermal layer will be found to consist of cells with large clear lumina, and thin walls of unaltered cellulose; they show no trace of suberization. An entirely different picture is presented by the hypodermal cells of the outermost living sheath. The walls are much thickened, having attained 4–13 μ in thickness where in the inner sheath they are less than 1 μ. They are penetrated by prominent radial pits and are distinctly laminate in places. The lumina are very much reduced. Microchemical tests show that these thick

walls are lignified. The anticlinal and inner walls of the epidermal cells are thickened and lignified, while the outer walls and the cuticle are apparently unaltered. Occasionally the walls of the cells of the layer next beneath the hypodermal layer are somewhat thickened and suberized.

The alteration of the hypodermal cells begins when the sheath, which is pushed from the interior toward the exterior of the pseudostem by the dying off of the old sheaths on the outside and the pushing up of new ones within, is fourth or fifth from the surface. The walls thicken gradually and become suberized,[6] lignification not occurring until a later stage, when the sheath is covered by only two or three of those exterior to it. The hypodermal cells bordering the substomatal chambers behave differently from the others, which alone are referred to in this description, in that their walls thicken and become lignified at an earlier period. Thus, for example, in the fourth sheath from the exterior they were found to be greatly thickened (as much as the other cells in the second or even the exterior sheath) and lignified, while the surrounding cells were very slightly thickened, and the chemical alteration had proceeded only as far as a faint suberization.

Since the alteration of the hypodermal cells commences in sheaths still tightly covered by those exterior to them, it is obviously not caused by their proximity to a transpiring surface. The penetration of sunlight is probably the stimulus initiating the changes in a given sheath. The first indications of the thickening and suberization of the walls (except of the cells bordering the substomatal chambers) are usually to be found in that sheath which is the innermost containing visible traces of chlorophyll, and there is at least a roughly quantitative correlation between the greenness of a sheath and the thickness of its hypodermal walls.

The hypodermal stratum of the inner surface of even an outer-

[6] That is, they show a strong retention of GRÜBLER's cyanin, stain yellow to brown with chloroiodide of zinc (with or without previous treatment in javelle water), are insoluble in conc. H_2SO_4, and do not stain in a solution of phloroglucin and HCl. Membranes are said to be lignified when they are insoluble in conc. H_2SO_4 and give a positive reaction with phloroglucin. They usually stain some shade of orange or brown in the chloro-iodide reagent. The same significance is attached to these terms throughout the remainder of this article.

most sheath is not greatly different from that of the inner sheaths. The walls of the former are faintly suberized, but not noticeably thickened, and never lignified. In this surface occur numerous small depressions or pits (fig. 5). These are circular to elliptical in outline, and vary greatly in size, the largest being 500 μ in diameter and 160 μ in depth. Their frequency is roughly 20–30 per $\overline{\text{cm.}}^2$ Both the epidermal and hypodermal cells beneath the pits are shallower than elsewhere, and small intercellular spaces containing air occur among the latter. The pit is lined with a cuticle perhaps slightly thicker than that found on the surrounding surface. When a section of the sheath, the cut ends coated with vaseline, is immersed in an aqueous solution of safranin or methylene blue, the stain penetrates and colors the whole inner surface, with the exception of the pits, which are impervious to it and remain white. The function of these pits, if any, remains problematical.

10. Vascular and mechanical systems

The ground tissue of the walls which border the lacunae of the sheath at their outer and inner ends and of the longitudinal septa is a thin-walled parenchyma, in which are distributed the vascular bundles and the strands of prosenchyma (fig. 17). The most prominent vascular bundles are those situated in the longitudinal septa. In the thicker portions of the sheath there are 2–4 large bundles in each septum, in addition to very few small ones, and occasional fibrous strands. A large bundle is situated in the outer wall opposite the center of each lacuna, and another where each septum joins this wall. Alternating with these is a series of smaller bundles, somewhat nearer the outer surface, and still a third series alternates with and is external to the second. Exterior to these are numerous small bundles, in which the vascular tissue is very much reduced or lacking, and the outermost bundles are reduced to mere strands of fibers. In the inner wall there are usually 1–3 small bundles opposite each lacuna, but the number may increase to 8 or 9 near the base of the sheath.

The development of the xylem, and in particular the fate of the protoxylem, present features of special interest and were studied in some detail. The course of the ascending sap in leaves of various

ages was traced by injecting the bundles with trypan blue in 1 per cent solution, as recommended by Miss BUCHHOLZ (2). Whole plants were cut from the bulb and set in the solution in a sunny place, where they were allowed to remain for six hours. In this period there was considerable upward movement of the dye, even through leaves still inclosed within the sheath and hence not transpiring, probably largely in response to the requirements of the tissues for growth.

The bundles at the inner edges of the longitudinal septa are those which show the largest series of protoxylem elements. The earliest and narrowest protoxylem elements have annular thickenings. Following upon these are tracheids in which annular thickenings may alternate with spiral bands. Next follow ducts thickened by a single spiral band, which in places may be doubled by splitting. Each of the successive protoxylem elements, of which there are 3–6 in the larger bundles, according to the bundle and the level of the cross-section, is of greater diameter than the last, until the single large metaxylem tracheid is reached. This tracheid is strengthened by 10–14 low-pitched spiral bands which divide and reunite, so that even in a single tracheid their number is not constant. This large element is succeeded toward the phloem by several narrow, spirally thickened tracheids which may briefly be designated the commissural connectives, since it is to them that the commissural tracheids are joined. The writer has distinguished as protoxylem all elements of temporary duration, and as metaxylem all which are permanently functional.

The spiral bands of these tracheids, like those of the tracheids in the stem, pull out readily, and a cutlass used in cutting up the banana plant (especially if it is not very sharp) is soon enveloped in a thick wad of them. WITTMACK quotes PACTON (15) as stating that this material once was employed as tinder in the West Indies.

As is generally known, the sheath of the banana leaf elongates through the activity of an intercalary growing zone situated in the region of its insertion on the stem. The rate of elongation of the leaf is very rapid, and MAXWELL (13) has recorded a maximum rate of growth of 21 cm. per day (see also TRELEASE 21). A cross-section just above the base of a sheath which is still growing shows that

in each bundle the 3–5 earliest protoxylem elements are still intact and stained with the trypan blue, indicating that they are active in conduction (fig. 14); several more protoxylem elements are in process of formation, and the large metaxylem tracheid is still small and unthickened. At a higher level the rings of thickening of these earliest elements have become more widely separated, and the surrounding parenchyma cells have begun to bulge into their lumina. The commencement of the process of occlusion of the earliest elements may clearly be recognized at 1 cm. above the insertion of the leaf. The metaxylem is still immature and does not function, but the younger protoxylem has matured to take the place of the disrupted earlier elements. At a still higher level the metaxylem begins to stain, and one or two protoxylem elements still conduct (fig. 15). The lacunae left by the disruption of the protoxylem continue to stain for some time after their occlusion by parenchyma cells has begun, and apparently play some part in conduction, although they soon become filled with a mucilaginous substance which stains deeply with Delafield's haematoxylin. Finally the lumina of the protoxylem elements are completely occluded by the inward growth of the neighboring parenchyma, and the metaxylem alone is functional. The isolated rings or elongated spiral coils are visible closely imbedded in the parenchyma (fig. 16). One large cell may more than cover the site of the vanished lumen. A longitudinal section shows that the smallest rings are very widely separated, and the distance between the rings or turns of a spiral decreases as the element to which they belonged lay nearer the metaxylem (was of later origin). The level at which the metaxylem begins to conduct depends upon the age of the leaf. In a leaf just appearing above the pseudostem the metaxylem did not stain at all, although the solution rose through the entire length of the sheath in the protoxylem; in another, which had spread its lamina but was still elongating, the metaxylem stained at 3 cm. above the insertion of the sheath.

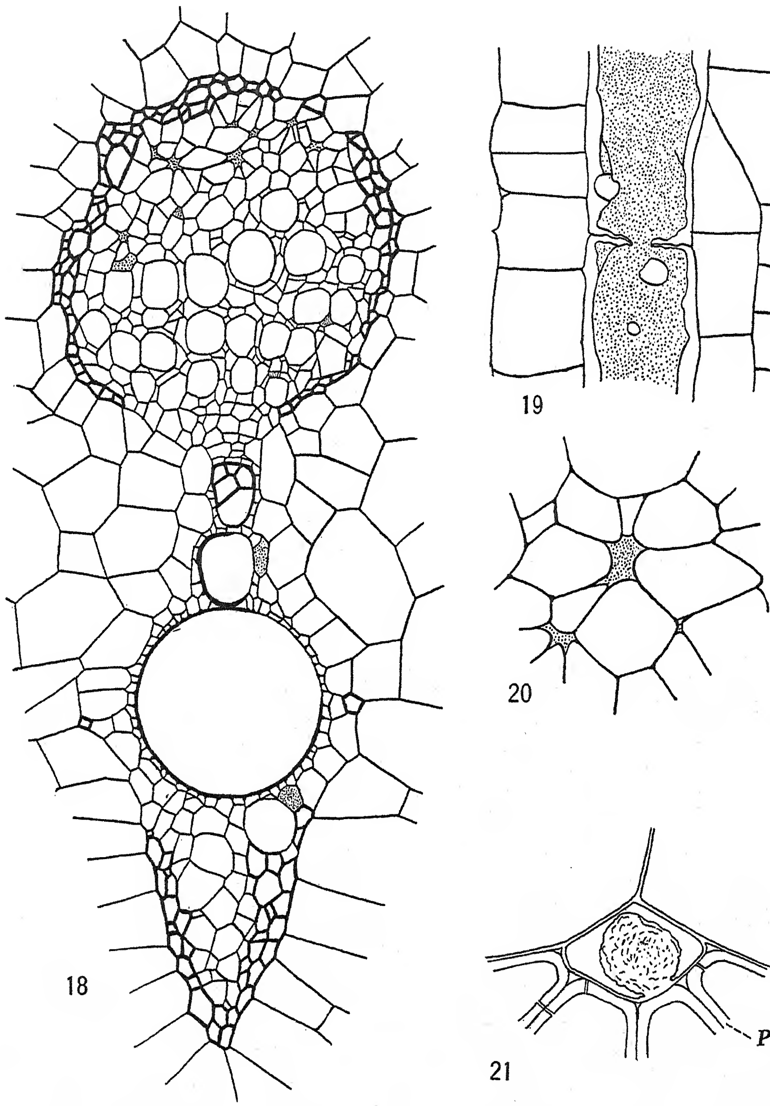
The sequence of events just outlined agrees essentially with that described for *Musa* by Miss Buchholz (2). *Musa* belongs in the third of the four classes of monocotyledons which she distinguishes on the basis of the fate of the protoxylem in organs with intercalary growth. If we trace the course of the ascending water current, we

find that it begins to rise in the innermost and earliest elements; as it passes upward it moves outward to younger and younger elements, following their wider lumina in place of the narrower ones which are becoming occluded. Finally the stream enters the large metaxylem tracheids, in which it continues up into the lamina.

In mature sheaths it is possible to cut sections passing between the widely separated rings which show no trace of the presence of the protoxylem, so completely is the scar healed (figs. 16, 18). The single large tracheid is very wide, reaching 0.25 mm. in the diameter of its lumen. According to HABERLANDT (9) the tracheids of *Musa* reach 1 cm. in length. The smaller bundles in the septa, and the inner and outer walls, do not exhibit a sequence of protoxylem elements, and are not functional in the intercalary growing zone. The bands of thickening of both protoxylem and metaxylem are suberized, but only occasionally does the metaxylem give a faint lignin reaction.

The cross-sectional area of the phloem is considerably greater than that of the persisting xylem. The sieve tubes (fig. 18) are large and conspicuous, and arranged with some regularity. The larger are 65 μ in diameter and 2.4 mm. long. The sieve plates are transverse or very slightly oblique. The lateral walls of sieve tubes, companion cells, and cambiform cells are penetrated by small circular pits. In immature bundles the walls of the sieve tubes are very noticeably thickened, but they become thinner as the phloem matures. In the more peripheral portions of the bundle small schizogenous slime passages are formed. These are surrounded by 4–7 cells which bulge into the intercellular cavity, and are filled with a mucilage which stains deeply (fig. 20).

The xylem and phloem of the larger bundles are each partially surrounded by a separate horseshoe-shaped sheath of mechanical tissue. The gap between the two rings is always opposite the commissural connectives, and through this break in the sheath the commissural bundles enter the longitudinal bundles (fig. 18). The thickness of the sheath depends upon the position of the bundle. In the large bundles in the longitudinal septa it is 1–3 cells thick. The relative development of prosenchyma increases in proportion as the bundle lies closer to the outer surface of the sheath. At the same time



FIGS. 18–21.—Fig. 18, vascular bundle from mature sheath; ×100. Fig. 19, immature mucilage vessel from petiole, showing perforation of end wall between two of cells composing vessel; ×345. Fig. 20, schizogenous mucilage passage from phloem of petiole; ×325. Fig. 21, stegma from bundle in sheath; *P*, prosenchyma; ×675.

the amount of conducting tissue is diminished, and the outermost bundles are mere strands of thick-walled fibers, without vascular tissue, which are largely responsible for the rigidity of the pseudostem. The sheath around the large interior bundles of the septa, especially its xylem portion, is often of rather thin-walled cells, and does not extend around the large tracheid. This is true especially at the base of the leaf sheath. Fig. 18 shows a sheath of moderately thin-walled cells incomplete around the large tracheid. The thinner fibrous sheaths are interrupted by gaps in which the cells are thin-walled, and serve as transfusion cells.

The composition of the walls of the fibers varies considerably in different leaves, and even in different portions of the same leaf sheath. The strands of fibers alone and the thick sheaths of greatly thickened cells in the outer wall of the leaf sheath are usually lignified. The thinner sheaths around the large inner bundles often give a distinct lignin reaction with phloroglucin, but in other places are suberized and not lignified.

FIG. 22.—Portion of tracheid from commissural bundle of sheath.

Accompanying the mechanical sheath on its outer face are groups of cells containing concretions of silica, the so-called stegmata. The stegmata are small cells with unthickened walls; they are short with blunt ends, and arranged in longitudinal rows. Each contains a roughly spherical mass of silica, apparently devoid of any organic basis (9), which almost completely fills the lumen, and is attached to the wall of the cell by a short stalk (fig. 21).[7]

Surrounding the bundles, outside the mechanical sheath, is a jacket of starch-filled parenchyma cells. This starch sheath persists even where it is separated from the vascular tissue by a sheath of 4–6 layers of heavily lignified fibers. The orientation of the principal bundles of the sheath, petiole, midrib, and lamina is always normal, the xylem toward the adaxial, the phloem toward the abaxial side.

[7] They may best be observed by following a modification of the method described by KÜSTER (11). Hand sections are dehydrated in alcohol and then mounted for observation in benzol, which gives the concretions a faint red tint. The cell walls may be stained in safranin, etc., while in the alcohol.

The curvature of a septum in the midrib may sometimes cause an easily detected physical reversal of morphologically normal orientation. In the longitudinal septa of sheath and midrib the axis of some of the smaller bundles without protoxylem is sometimes turned sideways, and occasionally the orientation is completely reversed.

The longitudinal bundles are connected at all levels by numerous commissural bundles, which lie in the plane of the horizontal dissepiments. However, only extremely rarely do these commissures strike across the dissepiment; normally their course is around its margin, where it joins the longitudinal walls of the lacuna. Here the commissural bundle may extend around two or three sides of the lacuna, sending out arms which connect with the small tracheids (commissural connectives) of the longitudinal bundles near its course. Other commissures occur in the outer wall. The commissural bundles consist of a small amount of phloem, and usually a single narrow tracheid, the thickening of which is reticulate and often of very intricate pattern (fig. 22).

11. Mucilage ducts and cells

External to the bundles but parallel to them are numerous mucilage ducts. These arise by the end-to-end union of a longitudinal series of parenchymatous cells, and correspond to the latex vessels of Haberlandt (9). Often several mucilage-bearing cells lie end-to-end, apparently without connection between them; at other times a large central pore penetrates the end walls separating the cells, and in some cases this wall almost completely disappears. A stage in the fusion of two mucilage cells to form a vessel in a young petiole is represented in fig. 19. Isolated cells having contents giving the same staining reactions as those of the mucilage ducts occur throughout the leaf, including the vascular bundles, and especially the phloem, where the cambiform cells may contain mucilage (fig. 18). In the lamina, sieve tubes which themselves contained a similar substance were discovered. The schizogenous passages in the phloem in all regions contain the same substance, and probably have a similar significance to the plant (fig. 20). The mucilage ducts are the *Milchsaft-Gefässe* of Wittmack. According to this author, their contents, in *M. ensete*, are rich in potassium chloride, potassium

oxalate, and tannins. The sheath of the Gros Michel is also rich in tannins.

12. Aerenchyma and air chambers

The lacunae arise by the tearing apart and destruction of the tissues of certain regions in the interior of the sheath; they are rhexigenetic in the sense of De Bary. A cross-section through a young sheath near its base shows regions of comparatively large cells, which have begun to round off, separated by smaller, closely packed cells. The former are in the lacunar region, the latter will become the longitunal septa. A longitudinal section through the lacunar region at a slightly higher level reveals transverse layers of intact cells separated by thicker layers of large, thin-walled cells devoid of much contents. The former give rise to the transverse septa, the latter are being pulled apart by the elongation of the sheath to form the lacunae. The disrupted tissue is evidently resorbed, since the mature lacunae are quite free from cell fragments. The dissepiments are of two kinds. Both contain the characteristic aerenchyma (fig. 6), made up of a reticulum of stellate cells. Morren (14) describes and figures the formation of the stellate cells. The septa of the first type are formed by 1–4 horizontal layers of this stellate aerenchyma alone. Those of the second type are composed of a single or several layers of small parenchyma cells situated between an upper and a lower stratum of the stellate cells, each of which may be one or two cells in thickness. The thick septa are more common in the sheath than in the midrib. Starch grains are numerous in both types of cells, and according to Greve (8) the transverse septa, particularly the central layer of parenchyma of the compound type, are a principal tissue for the storage of starch. The lacunae are usually filled with air, but in wet weather may become partially or almost completely filled with water, a circumstance noticed also by Baccarini (1, fide Greve).

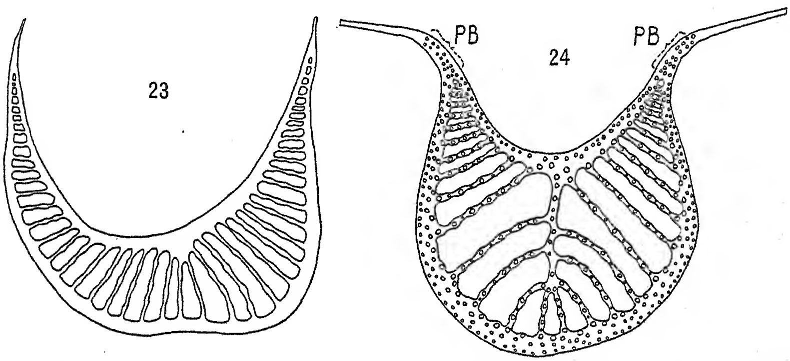
Lying free on the surface of the transverse septa are numerous large raphid cells filled with bundles of acicular crystals of calcium oxalate. These cells are furnished with processes resembling those of the star-shaped aerenchyma, and by these they are attached to the latter. They may attain the very great size of 322 μ in length and 81 μ in diameter. Raphid bundles are numerous in giant cells

imbedded in the tissues of all portions of the leaf, and isolated monoclinic crystals of calcium oxalate are abundant in the parenchyma everywhere.

B. PETIOLE AND MIDRIB

13. Gross structure

The petiole and midrib resemble each other in general structure so strongly that they may be described together, occasional differences being noted where necessary. At the top of the pseudostem each sheath begins to become narrower and thicker. The central



Figs. 23, 24.—Fig. 23, cross-section at about middle of petiole, showing transition from structure of sheath to that of midrib; ×0.5. Fig. 24, cross-section of midrib, near base of lamina of large leaf, position of principal vascular bundles indicated by circles; *PB*, pulvinar band; ×1.

lacunae contract toward the adaxial surface; they are crowded inward by those lateral to them, and these finally meet in the central line, overarching the central lacunae and pushing them away from the adaxial wall (fig. 23, and cf. figs. 17 and 24). This change from the typical structure of the sheath to that of the midrib occurs gradually in the proximal portion of the petiole, and the typical cross-section of the midrib is presented by its distal portion. This structure will be sufficiently clear from fig. 24. It is only necessary to add that the midrib, which near its base is very massive in large leaves, 4.5 cm. from side to side and 2.7 cm. from top to bottom along the median line, tapers gradually toward the apex, the lacunae becoming fewer and smaller. It does not, as in many monocotyledons

(for example, the related *Strelitzia reginae*), blend into the blade before the apex of the leaf is reached, but is distinct throughout the length of the latter (fig. 12).

14. Peripheral tissues

As in the sheath, the peripheral tissues on the two sides of the petiole and midrib differ markedly. Considering first the lower surface, the epidermal cells are of the same general type as those of the sheath, but shorter. A thick external wall of cellulose is covered

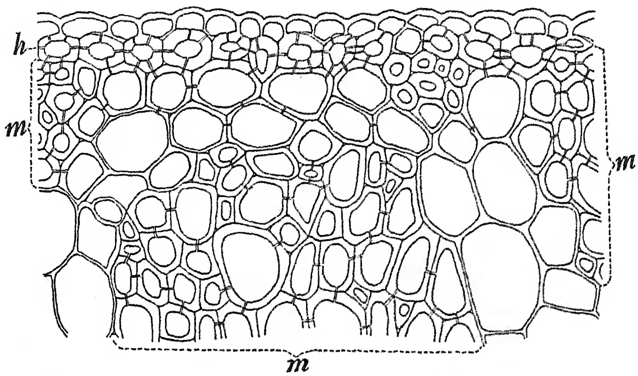


Fig. 25.—Peripheral region of lower side of midrib; *H*, lignified hypodermal layer; *M*, fibrous strands; ×350.

by a thin cuticle, which bears a very heavy waxy "bloom," similar to that of the lamina mentioned later. The hypodermal layer is made up of cells rectangular in surface view, their long axes coinciding with the length of the midrib. These cells are very thick-walled and lignified, and their walls are penetrated by prominent radial pits. Beneath this is a second layer of cells with walls slightly thinner, but also lignified and pitted. Within these are 2–3 layers with walls slightly thickened, and suberized rather than lignified (fig. 25). The lignification of the walls of the hypodermal cells becomes incomplete, and that of the second layer disappears near the distal extremity of the midrib. The continuity of all strata interior to the hypodermal layer is broken by numerous strands of lignified prosenchyma. These fibrous bundles are very abundant in this region, the outermost abutting on the hypodermal layer itself. They are of all

sizes, ranging from 5–6 to several dozens of cells in cross-section, and they lie so close together that usually not more than three cells of the ground tissue intervene between two adjacent strands (fig. 25).

The upper surface bears an epidermis covered with a moderately thick cuticle, reaching 2 μ in thickness. Beneath this are 2–3 layers of large colorless cells, elongated from side to side of the midrib, forming a hypodermal water tissue. Their general appearance in surface view resembles that of the corresponding cells of the lamina, reproduced in fig. 32. Except at the edges of the midrib, near the pulvinar bands, the walls of these cells are slightly thickened and suberized, but not lignified as in the case of the lower surface. The fibrous strands are not nearly so numerous beneath the upper as beneath the lower epidermis.

15. Internal anatomy

The distribution of the vascular bundles between the walls and the longitudinal septa resembles that of the sheath, and is shown diagrammatically in fig. 24. The general character of the bundles is much the same as in the sheath, and the reaction of the cell walls of corresponding elements is similar. The larger bundles show the same succession of occluded protoxylem elements, but the process of occlusion was not studied here. As hinted previously, the fibrous strands in both outer and inner walls, as well as in the septa, are more numerous than in the sheath. These strands are usually but not always lignified, but if not lignified at least they are suberized. The commissural bundles are of the same type as in the sheath, but more often pass directly across the transverse septum from side to side of the lacuna, instead of hugging the longitudinal walls. When the petiole and lower portion of the midrib are split in half longitudinally, and one side only placed in a solution of eosin, the solution readily crosses over in the commissural tracheids, and moves downward into the midrib and blade of the other half below the upper end of the cut (50.cm. below in one experiment which lasted 3 hr. 20 min.). The transverse septa are of the same type as in the sheath, but the thinner kind, composed of aerenchyma alone, without the small-celled central parenchyma, are relatively more numerous here.

It may be profitable to consider for a moment the mechanical features of the midrib and petiole, which must support in an almost horizontal position an enormous expanse of lamina. The lower surface, the term extended to include all of that portion beneath the pulvinar bands, is surrounded by a woody shell at least two cell layers thick, strengthened on the inner side by numerous longitudinal ribs, the fibrous strands. This woody shell with its supports, because of the curvature of its walls, is well qualified to resist buckling under the compressional stresses to which it is subjected. The prosenchymatous strands, both surrounding the vascular tissue and distinct from it, concentrated in the upper wings of the midrib and petiole, are adapted to support the tension to which their location exposes them, and are more efficacious here than an equal number would be if collected in the central portion of the upper wall, since in their actual position they are farther removed from the lower side.

16. Pulvinar bands

The diurnal movements of the lamina were described by Johow (10) and later by Trelease (20). In the early morning, or during a wet day, the two halves of the lamina stand out almost in a horizontal plane on either side of the midrib (fig. 27). During a bright day the lamina halves bend downward, bringing their lower surfaces together beneath the midrib (fig. 26). Where the lamina has been torn into strips, the individual segments move together synchronously, almost as though the lamina were intact. This movement occurs rapidly during the morning of a dry day, and undoubtedly is very effective in decreasing transpiration, since most of the stomata are on the lower surface, and the profile position assumed greatly diminishes the amount of radiant energy absorbed. The change in the appearance of the plant is very striking. The movement is not a passive drooping brought about by the wilting of the leaves, but is produced by the activity of the pulvinar bands, which bend downward along the sides of the midrib in response to changes in the turgor of their tissues.

The pulvinar bands lie along the edges of the midrib, flanking it for its entire length, and merge on the external side into the blade (fig. 24 *PB*). They differ from the tissues on either side of them in

that they contain no lacunae. Their thickness is between 1.2 and 1.8 mm. The ground tissue is of rounded parenchyma cells, resembling the motor tissue of the pulvini of dicotyledonous leaves. Beneath the epidermis is a water tissue composed of 4–6 layers of clear

FIG. 26.—Banana plant photographed at noon on a bright day; "false pinnae" hanging downward in profile position.

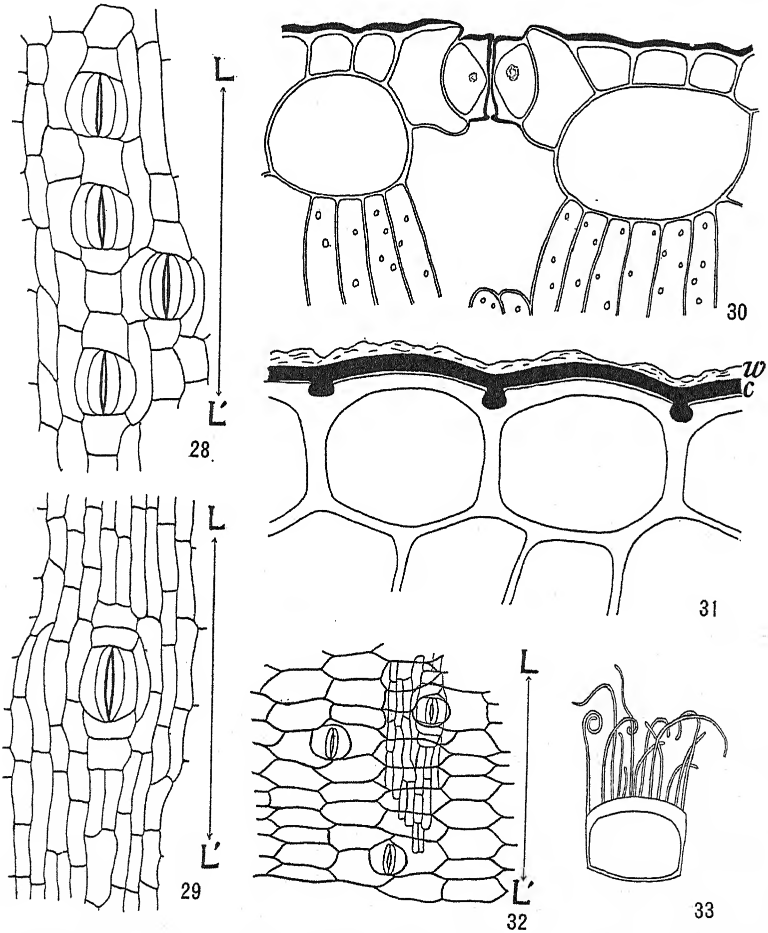
cells. Next below this is a chlorophyllous layer of close set, rounded (not palisade) cells. This borders below a region of thin-walled, rounded parenchyma cells, containing little chlorophyll, which occupies the central portion of the organ. In this tissue are situated the vascular bundles, which here are surrounded by a very thick mechanical sheath, probably an adaptation to prevent injury to the

vascular elements by the bending of the pulvinus. The small metaxylem elements (commissural connectives) are much more numerous in this region than elsewhere in the leaf, and several of the large tracheids may occur in the cross-section of each bundle. Anasto-

FIG. 27.—Same plant as fig. 26, just after sunrise following morning; "false pinnae" standing out almost horizontally.

moses between the bundles are frequent. Below the central parenchyma are 2–3 layers of large, upright prismatic cells, in which run a few fibrous strands. Abutting on the lower epidermis is another water tissue of 2–3 layers of clear cells, slightly thick-walled, elongated in a direction parallel to the midrib. The exact mechanism of the movements of the organ and the functions of the various tissues have not been determined.

A peculiarity of the cuticle over the upper epidermis deserves mention. Like that over the rest of the upper surface of the lamina, this cuticle is rather thick. Distinct knobs project from its inner



FIGS. 28–33.—Fig. 28, lower epidermis of lamina in surface view (line *LL'* drawn parallel to veins); ×315. Fig. 29, upper epidermis; ×315. Fig. 30, transverse section through stoma and accessory cells of upper epidermis, cuticle indicated by heavy black line; ×570. Fig. 31, upper epidermis from pulvinar band of mature leaf; *C*, cuticle; *W*, waxy layer; from a section stained with Grübler's cyanin and aqueous safranin; ×1100. Fig. 32, upper hypodermal layer, as seen from inner surface, showing also some of cells of upper epidermis; ×135. Fig. 33, rods of wax forming the bloom, from lower epidermis of leaf just expanded; ×about 800.

surface where it overlies the anticlinal walls of the epidermal cells, and penetrate the latter, the cellulose wall, which is here rather thick, being hollowed out to receive them (fig. 31). Often the knob is distinctly constricted at the neck, and the epidermal wall narrows around the constriction. The cuticle is thus anchored to the epidermis by a ball-and-socket device. In portions of the cuticle isolated by treatment with concentrated sulphuric acid to destroy the cellulose, the knobs appear as a series of distinct, equidistant projections which outline the epidermal cells. Often there appears to be a narrow gap between the epidermis and the cuticle over the middle of an epidermal cell. The cuticular knobs are confined to the upper surface of the pulvinar bands and the portion of the midrib immediately adjoining. In other regions the cuticle comes down in points over the anticlinal walls of the epidermal cells, but is not knobbed. The arrangement appears to be associated with the motility of the pulvinar band, and perhaps aids in securing the cuticle to a surface which undergoes great changes in curvature.

C. LAMINA

17. External characters

The outline of the lamina is ovate-oblong. The apex is blunt, but the two sides are unlike (fig. 12). The left half, which is external in vernation, extends farther down the petiole than the right (fig. 9), and in large leaves this difference may amount to 12 cm. WITTMACK states that in *M. ensete* the covered half extends farther down the petiole than the one which is external in vernation, and is likewise markedly broader (external half 41 cm., internal 50 cm.). In many species of the Marantaceae, Musaceae, and Araceae, it seems to be a general rule that the half of the lamina which is covered in vernation is broader than the exterior half, often (as in *Calathea* spp., *Musa cavendishii*, etc.) pronouncedly so. The general rule holds in the case of the Gros Michel, but the difference between the halves is not so great. In the few leaves of greenhouse plants available, the right side was 2–10 per cent broader than the left.[8]

[8] The writer is indebted to P. R. WHITE for measurements of leaves growing at the New York Botanic Gardens. He neglected to observe this point during his sojourn in Jamaica.

The thickness of the blade increases gradually from margin to midrib. The difference is quite considerable, and a large leaf may be 3.5 times as thick next to the midrib as at the margin. Both the

TABLE II

INTERNAL TOPOGRAPHY OF A LAMINA 340 CM. LONG

POSITION		MEASUREMENTS			
Distance above base (cm.)	Distance from pulvinar band (cm.)	Thickness of lamina (μ)	Depth, upper epidermis + water tissue (μ)	Depth of palisade tissue (μ)	Depth of lacunae (μ)
10	0	718	68–91	76–95	....
	0.6	568	76	91	317
	1.2	518	65	95	267
	2.5	484	61	99	251
	5.0	434	61	95	217
	10.0	351	57	87	134
	16.0(M)*	267	57	76	57
120	0.0	935	76	122	601
	1.9	752	57–76	118	484
	3.8	685	53	118	418
	7.5	618	49	114	351
	15.0	501	53	110	267
	30.0	367	38–53	95	156
	44.0(M)	267	42	84	57
220	0.0	768	65	122	484
	1.6	618	49	122	367
	3.3	585	53	114	317
	6.5	534	49	110	284
	13.0	451	46	106	234
	26.0	334	27–46	95	134
	39.0(M)	251	38–57	84	57
330	0.0	534	57	106	251
	0.6	468	57	95	217
	1.2	434	57	95	200
	2.3	401	53	95	184
	4.5	384	30–53	95	167
	9.0	317	30–53	91	134
	14.0(M)	301	53	80	80

* M = margin.

absolute and proportional increase in thickness are greater in the middle region of the leaf than at the base or apex. The rate of increase in thickness is not uniform from margin to midrib, but becomes greater as the midrib is approached (table II). As is apparent from the table, the increase in thickness is accounted for largely

by the increase in depth of the lacunar region, the palisade and dermal regions changing relatively little.

From the strong midrib the pinnately arranged veins follow an S-shaped course to the margin. All of the veins of the same region of the leaf are very nearly parallel. The branching of the bundles and the outward curvature of the S occur principally in the pulvinar band, and the forward curvature does not assume prominence until the vein has approached to within 5 mm. of the margin, so that across the breadth of the blade the veins follow a course which is practically straight, except at the extreme base and apex of the lamina. At their distal extremities the veins bend forward and unite in the marginal bundle, which practically surrounds the lamina. The apparent parallelism of the veins is not exact. At the base of the lamina they are directed backward as they leave the pulvinar band, forming an angle of 100°–120° with the midrib. At the apex their course is forward, the angle becoming 55°–77°, while the middle veins are nearly perpendicular to the midrib. As the result of this, all of the veins diverge slightly, and any strip across the blade, such as that torn by the wind, is wider at the margin than at the midrib.

Two orders of veins are apparent upon the most superficial examination. The strong or principal veins lie at the apex of an upward curvature of the lamina, and accordingly are situated above its general surface. This fold gives the lamina a ribbed appearance (fig. 12), which is in nowise caused by the vein itself, for this is not appreciably thicker than the immediately adjoining portions of the blade. The weak or subordinate veins lie flat in the plane of the blade. The anatomical differences accompanying these superficial distinctions are discussed later. Both the strong and weak veins exhibit great differences in development, and there are gradations between the two. Alternating with the strongest veins there is usually a somewhat weaker vein of the same character, easily distinguished from the subordinate veins lying between them. WITTMACK distinguishes six orders of veins in *M. ensete*, and presents a diagram showing their derivation from a single bundle. Since the number of subordinate veins between each pair of principal veins is not constant, it is probable that in the Gros Michel there is no very definite scheme of ordination.

Practically all of the veins which extend into the blade from the midrib continue until they reach the marginal bundle, or stop within 1.5 cm. of the margin. However, new subordinate veins may arise blindly between any pre-existing pair of them in any region of the blade, and they have been observed to begin less than 1 cm. from the margin. The intercalated veins do not arise by the branching of the original veins; their connection with the vascular bundles of the midrib is by means of the commissural bundles linking them with adjacent veins. The length of the intercalated veins may be less than 1 cm., in which case the vein ends blindly as it began, but the majority run out into the marginal bundle. As a result of this intercalation of veins, there are more subordinate veins between each pair of principal ones at a few centimeters in from the margin than near the midrib. The increase in the number of veins by intercalation is greatest at the base of the lamina, where the average increment in the two leaves for which counts were made was 36 per cent. At the middle of the leaf the increment was 20 per cent and at the apex 9 per cent. Because of the intercalation of veins, all regions of the lamina are equally well supplied with vascular bundles. The average distance between subordinate veins is practically the same at the margin as at the midrib, the variation in average separation amounting at most to 0.03 mm. The interval between veins is very nearly the same in all regions of the lamina, the averages lying between 0.19 and 0.26 mm. The average distance between the strongest principal veins is about 1.3 cm., but great variation is found here. Usually between 35 and 60 subordinate veins, as well as the weaker principal vein, occur between each pair of the strongest veins, the count being made at the midrib. The number may rise to 100, however, in large leaves. Taking 0.24 mm. as the average interval between veins, a lamina 394 cm. long would have about 17,000 veins on each side near the midrib, and correspondingly more near the margin.

One other feature in the external topography of the lamina seems worthy of notice. On many leaves one can distinguish longitudinal streaks running along the lamina parallel to the midrib, often giving a faint suggestion of ribs (faintly visible in figs. 12 and 13). The ribbed appearance is due to nothing more than kinks in the blade, slight furrows being impressed upon the upper surface, with

corresponding low narrow ridges upon the lower, although occasionally the direction of the curvature is reversed. The furrows are conspicuous chiefly in consequence of their great length, stretching from end to end of the lamina. These furrows are associated with no structural differentiation of the blade, but result from unequally distributed pressure upon the young leaf still rolled within the pseudostem, causing folds which are not entirely lost by the lamina in expanding. The number and distribution of the furrows on the two sides of the lamina lend weight to this view. Generally there are 14–20 on the right half at its widest part, and 4–5 on the left, although it is difficult to give figures, because some furrows are so indistinct that one is at a loss to decide whether they should be considered as such or not, and there is great variation between individual leaves. The spacing also differs on the two halves. On the right half the furrows are very close together near the margin, and the interval between successive furrows increases as the midrib is approached. On the left half the 4–5 furrows are almost equidistant. These two circumstances agree with the number and diameter of the coils in vernation (see Section 7). The most distinct furrows are near the right margin of the leaf, corresponding to the narrower coils in this region.

In vernation the coiled lamina usually does not show two perfect helices in cross-section, but, especially in the later stages, the inner helix is generally flattened in the plane of the expanded lamina (fig. 8). In some cases the coils may be flattened like cloth rolled around a board, and even folded back upon themselves in the form of a V. This departure from the ideal form in vernation is responsible for the permanent disfigurement of the tissues of the right half of the lamina. The furrows on the left half probably result from pressure exerted on the blade where it crosses the keel of the midrib in vernation, this being rather sharp at certain stages.

18. Dermal system of lamina; number of stomata

Both the upper and lower epidermis are composed of straight-walled cells, almost rectangular in surface view, elongated in a direction parallel to the veins (figs. 28, 29). The upper epidermis is covered by a rather thick (1–2 μ) cuticle, while that over the lower

is considerably thinner (0.5 μ or less). The lower surface of the lamina is covered by a thick "bloom" which gives it a glaucous color. In leaves just expanded it may be seen that the "bloom" is formed by innumerable close-set rods of wax, which are very slender and often curl up at the free ends (fig. 33). The rods soon crumple and slump together, and in old leaves they are represented only by irregular waxy masses. The "bloom" on the young leaves is so thick that it can be rubbed off on the fingers in large quantities as a white powder.

The stomata are all oriented with their long axes parallel to the veins and to the length of the epidermal cells. On the lower surface, where they are most numerous, they lie over the lacunae in narrow bands stretching across the blade from midrib to margin, while the adjacent strips of epidermis over the veins are almost devoid of them. The stomata of the upper epidermis are somewhat larger than those of the lower, the guard cells of the former measuring about 32 μ in length, those of the latter 28 μ, and other dimensions are in proportion (cf. figs. 28 and 29). The ventral ridges are not very pronounced, and the front and back cavities of the pore are small or indistinct. The cutinization of the guard cells is indicated in fig. 30. The passage between the guard cells is lined with a cuticle which extends over the inner periclinal walls, and over the outer walls is continuous with the general cuticle of the epidermis. The guard cells are bordered by accessory cells (figs. 28, 29, 30), and the short broad cells at either end of the stomatal group have a characteristic shape. The thick outer walls of the accessory cells are constricted to a more or less pronounced hinge where they join the guard cells. The statement of SCHUMANN (20) that the rods of wax which make the "bloom" on the lower epidermis bend over the stomata to form *einem oben offenen Dom* seems to the writer to convey an exaggerated idea of the definiteness of the structure in question and the part played by it in reducing transpiration. Individual rods arising from neighboring cells do bend over into the free space above the guard cells, but, in the Gros Michel at least, are arranged to form no definite structure.

The average of 35 separate determinations of the number of stomata per sq. mm. of the lower surface of the lamina is 168.5.

The variability in different leaves, and different regions of the same lamina, is so great that the figure just given has little significance. Individual counts (of an area of about 0.7 $\overline{\text{mm}}.^2$) varied from a maximum rate of 261 per $\overline{\text{mm}}.^2$ to a minimum of 96 per $\overline{\text{mm}}.^2$ Certain definite tendencies of distribution were manifest in all of the

TABLE III

DENSITY OF STOMATA ON LAMINA

Region	Number of stomata per sq. mm.					
	Lower surface*			Upper surface†		
	Midrib	Middle	Margin	Midrib	Middle	Margin
Base	134	169	143	36	31	26
	Mean for base = 149			Mean for base = 31		
One-fourth up	223	229	128	69	55	34
	Mean = 193			Mean = 53		
Half-way up	184	214	114	87	62	39
	Mean = 181			Mean = 63		
Three-quarters up	232	242	224	78	66	44
	Mean = 233			Mean = 63		
Apex	259	261	182	50	54	56
	Mean = 234			Mean = 41		
Mean	206	223	158	64	54	40

* Lamina 391 cm. long, 104 cm. in greatest width. Stomata in an area of epidermis 0.686 $\overline{\text{mm}}.^2$ counted in each case.

† Lamina 353 cm. long, 97 cm. in greatest width. Stomata in an area of 1.372 $\overline{\text{mm}}.^2$ counted in each case.

leaves of which a series of counts was made. Thus the basal quarter of the lamina has fewer stomata per unit area than those portions distal to it (table IV).

TABLE IV

Average no. of stomata at base of lamina	124.1 per $\overline{\text{mm}}.^2$ (maximum 169, minimum 96)
Average no. of stomata in central half of lamina	174.8 per $\overline{\text{mm}}.^2$ (maximum 240, minimum 97)
Average no. of stomata at apex of lamina	170.6 per $\overline{\text{mm}}.^2$ (maximum 261, minimum 117)

Ratio, central half 1.41 : apex 1.37 : base 1.00

In every leaf the average density in the central and apical portions was greater than the maximum density found at the base of the same leaf. Likewise there are more stomata in the region of each blade near the midrib than in that near the margin, except at the base of the lamina, where the order seems reversed (table V).

TABLE V

Average no. of stomata near midrib	185.2 per $\overline{\text{mm}}.^2$ (maximum 259, minimum 145)
Average no. of stomata near margin	148.9 per $\overline{\text{mm}}.^2$ (maximum 224, minimum 97)

(Counts at base of lamina not included in average)
Ratio, midrib 1.24: margin 1.00

The maxima and minima overlap because some leaves have everywhere more stomata than others. When the counts at the margin and the midrib of a given level of the same lamina are compared, the midrib exceeds the margin in every case except at the base. There are some indications that the density of stomata midway between midrib and margin is greater than in either the interior or marginal region of the blade (table III). The individual counts of a representative leaf are recorded in this table.

On the upper surface the average based upon 28 counts is 40.1 stomata per sq. mm. The maximum density found was 89 per $\overline{\text{mm}}.^2$, the minimum 3 per $\overline{\text{mm}}.^2$ Here again the basal portion fell behind the more apical regions in number of stomata. The ratios of frequency were central half 1.83: apex 1.75: base 1.00. The stomata are again more numerous in the central than in the marginal region of the lamina, and the difference is even more pronounced than in the lower surface, the ratio being midrib 1.51: margin 1.00. Finally, it may be recorded that large leaves near the crown of the plant seem to have more stomata per unit of area (both surfaces) than smaller leaves from lower down.

A larger number of determinations might have been made, but since the tendencies of distribution just recorded repeated themselves on every leaf with much constancy, these conclusions are stated with a fair degree of confidence, although the figures given are of value only as examples of what differences are to be expected. There would be little point in presenting those obtained in greater detail. Per-

haps their principal worth is to serve as a caution to those who might attempt to employ large leaves, such as that of the banana, in experiments in which the rate of transpiration or assimilation is to be correlated with the number and condition of the stomata. WITTMACK's figures on the number of stomata of the leaf of *Musa ensete*, 260 per $\overline{\text{mm}}.^2$ on the lower surface, 7 per $\overline{\text{mm}}.^2$ on the upper surface, are given here for comparison with those of the Gros Michel.

Beneath both the upper and lower epidermis there occurs a water tissue of thin-walled cells with clear contents (figs. 30, 35, 36, etc.). The cells in this tissue lie with their long axes parallel to the midrib and transverse to the veins; they are crossed with the epidermal cells (fig. 32). The thickness of the water tissue varies according to the region of the lamina. The upper water tissue is usually two layers thick just within the marginal bundle, somewhat farther in it is often single-layered, but usually double, near the midrib it becomes triple, and still closer it is often quadruple. The transition from the double to the triple condition is illustrated in fig. 36. The tissue is usually thicker where it overlies a vein than in immediately adjacent regions. The lower water tissue is often double near the margin, and double or triple near the midrib; over most of the lamina it is single or sparingly double.

Where one of the stronger of the longitudinal ribs just described crosses one of the principal veins, a node or swelling, paler in color than the rest of the lamina, may often be seen upon the upper surface of the leaf. Since a node at these places is not of constant occurrence, and they are absent from many ribbed leaves, the swelling is obviously not the cause of the rib, but rather a result of its presence. It is caused by a hypertrophy of the cells of the water tissue at the points in question, which elongate enormously in a direction normal to the surface of the lamina. Where the water tissue is two-layered only the cells of the inner layer elongate, and where it is three-layered only the inner two, the outermost layer remaining normal and being pushed passively upward. Not only the cells above the principal vein, but those on either side, often passing over several subordinate veins, exhibit the hypertrophy (fig. 34). Cells of this character may reach 317 μ in depth, about thirteen times the normal dimension, and a three-layered water tissue, of which the two inner

layers have hypertrophied, may become 568 μ thick. The hypertrophy at the intersection of vein and rib occurs only in the case of furrows upon the upper surface; where the upper surface is ridged, the swelling is manifest along that portion of the vein between the ridges. Hypertrophy of the water tissue over a principal vein may occur close to the midrib at points where it is not associated with a longitudinal furrow.

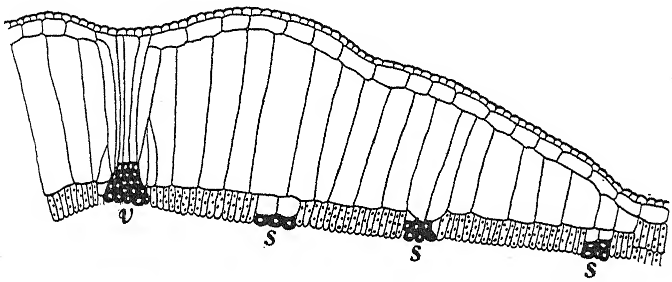
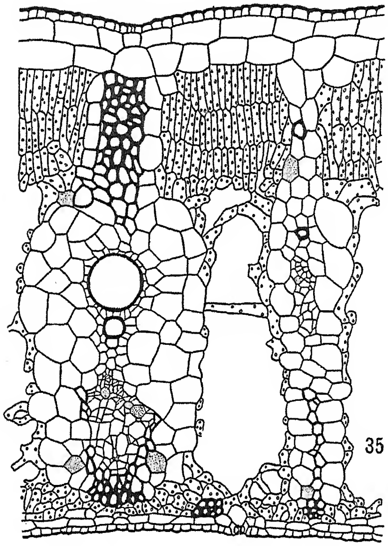


FIG. 34.—Hypertrophied cells of hypodermal layer at intersection of principal vein (*V*) with longitudinal furrow; *S*, subordinate vein; ×60.

18. INTERNAL TISSUES OF LAMINA

The interior of the lamina is divided by the veins into a series of canals which stretch from midrib to margin (fig. 35). Each canal is cut up by transverse septa into chambers or lacunae (fig. 36). The average length of the lacunae (the dimension parallel to the veins) increases markedly from margin to midrib. The average distance between transverse septa at the base of the lamina was 95 μ at the margin, 182 μ midway between margin and midrib, and 230 μ near the midrib. In the middle of the lamina the corresponding figures were 68–122–204 μ; near the apex 75–119–127 μ. On the contrary, the average width of the lacunae is greatest near the margin, where the veins are thinnest, and diminishes toward the midrib; it ranges between 170 μ and 73 μ. (For the depth see table II.)

Between the veins and above the lacunae lies the palisade tissue (figs. 35, 36), composed of three layers of distinct palisade cells, with frequent suggestions of a fourth. The palisade tissue is not interrupted by the transverse septa, and forms a continuous narrow band from midrib to margin. It is somewhat deeper near the



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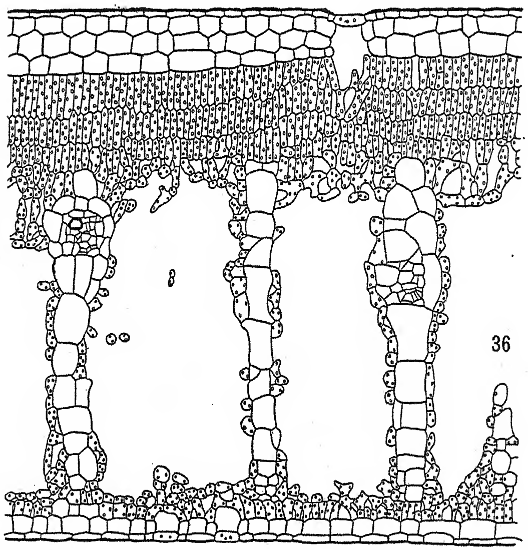

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FIGS. 35, 36.—Fig. 35, section of lamina transverse to veins, at about midway between margin and midrib; both veins of "subordinate" type; ×135. Fig. 36, section parallel to veins, septa to right and left being supplied with vascular bundles, while central septum is devoid of them; ×135.

midrib than at the margin, but its thickness does not increase nearly so rapidly as that of the lamina as a whole (table II). The lacunae are lined with spongy parenchyma, which occurs beneath the palisade and above the ventral water tissue, and borders the veins and transverse septa. Frequent elongated chlorophyllous cells, somewhat resembling the algiform cells of moss capsules, stretch across the lacunae.

The vascular bundles are surrounded by one to several layers of clear parenchyma cells, elongated parallel to them. The larger bundles contain a single large tracheid strengthened by twelve or less spiral bands, according to the size, and several smaller tracheids (commissural connectives) below them (fig. 35). The spiral bands of the xylem are suberized. The weakest bundles contain only a few very narrow tracheids; more rarely, near the margin, no xylem at all. The phloem is always present and more fully developed. The latter contains the same schizogenous mucilage cavities described for the sheath, and sieve tubes with the same dark-staining contents occur. Mucilage ducts are present in the parenchyma surrounding the bundles, and individual mucilage-filled cells occur in the phloem.

Accompanying all but the weakest veins are two strands of prosenchyma, one above and the other beneath the vascular bundle. The fibers in the lower strand are lignified, but those of the upper are suberized. The lower strand is accordingly more durable, and in portions of a leaf along a tear it persists after the upper strand has decayed. In the weakest veins, the upper strand is reduced to one or two isolated fibers in cross-section. In strong veins the lower strand extends out as arms which protect the phloem, but in weak veins this portion of the prosenchyma is reduced to a horizontal plate of cells one layer thick (as in the vein to the right in fig. 35). Strands of mechanical cells sometimes occur beneath the lacunae, apart from the veins, especially near the midrib, and at times are associated with a wall which projects upward into the lacuna, but does not reach the palisade tissue.

The principal veins, lying at the apices of the transverse folds of the lamina, are distinguished from the subordinate ones by a much greater development of the two mechanical strands accompanying them, and by the series of protoxylem elements which are dis-

rupted and occluded by neighboring parenchyma cells. The subordinate veins do not contain protoxylem. The stronger of the subordinate veins contain as much metaxylem and phloem as the principal veins in the same part of the leaf.

Except where a commissural bundle is present, the transverse septa are a single layer of cells in thickness, with a broken layer of spongy mesophyll on either side. The central cells have clear contents, and in surface view are very irregular in shape. The irregular projections and re-entrant angles interlock and a close tissue is formed. The mesophyll cells are elongated parallel to the wall, much branched, and very complex in outline. One, two, or three together of the central cells (in septa where there are no commissures) may become thick-walled mechanical cells. These cells are often very broad and long, stretching most of the way across the septum, and have the same irregular outline as the other cells of the central layer. The walls are penetrated by prominent pits. These mechanical elements are not numerous, but are most frequent near the margin of the lamina.

The commissural bundles which connect the veins run through the transverse septa. They consist of a single tracheid (in cross-section) and a small amount of phloem, but no mechanical elements, and resemble the commissures in the sheath and midrib (fig. 36). Their course is usually not horizontal, because the bundles of the veins which they join are not always at the same level. By no means every septum contains a vascular bundle, and as many as fourteen septa devoid of a bundle may intervene between two having them, although often only a single one intervenes. If the veins supplying a certain portion of the lamina are severed near the midrib, so that its supply of water must travel longitudinally through the commissural tracheids, and the petiole is then placed in solution of eosin, the commissural bundles are stained and stand out clearly when held up to the light. They appear as thin, tortuous red lines with frequent anastomoses, the general direction of which is parallel to the midrib. They are not distributed at random among the septa, but arranged as though with some regard to their continuity as water courses transverse to the veins. The commissures are often continuous across one or more veins; however, usually the septa equipped with com-

missures are not in line on the two sides of the vein, but one is displaced slightly in or out with reference to the other, so that the sap must move along the vein for less than the length of one lacuna to pass from one commissure to the other. Thus they do not form an independent system like the veins, and their continuity as a water course is dependent upon the latter. The apparent anastomoses occur where two commissures join a vein almost opposite a single one on the other side. The efficiency of the commissural bundles as water courses was demonstrated in many experiments, a few of which are recorded here.

1. A strip of the lamina extending 35 cm. in the direction parallel to the midrib, and about 15 cm. in the direction of the veins, was cut from a large leaf while the dew was still upon it. The severed ends of the veins were smeared with vaseline. The lower end was cut into fringes to increase the absorbing surface, and the strip (*A*) was hung with this end dipping into a 0.75 per cent solution of eosin. Another strip (*B*) from the same leaf was cut with the length perpendicular to the midrib and parallel to the veins, and hung in the same manner beside the first. The preparations were cut under water. The solution could ascend the strip *B* through the veins, but in *A* it could move upward only through the commissural bundles. The strips were left thus for four hours on a sunny morning (August 27, 1926). At the end of that time the solution had stained *A* 20 cm. above the lower end, while in *B* it had risen 34 cm., to within 1 cm. of the top, and probably would have gone farther had the strip been longer.

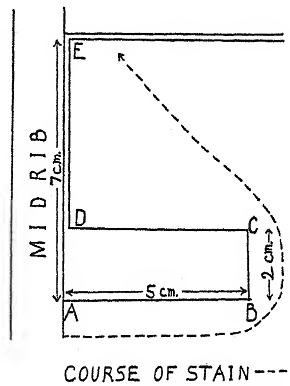


FIG. 37.—Diagram to illustrate Experiment 2.

2. A lamina was cut as shown in fig. 37. To reach the portion marked *E* it was necessary for the solution to travel 5 cm. outward along the vein from *A* to *B*, then 2 cm. upward through the commissural bundles from *B* to *C*, then inward through the veins and upward through the commissures from *C* to *E;* altogether at least 7 cm. upward through the commissures. The severed petiole was

placed in a 0.75 per cent solution of eosin at 9:00 A.M., and the experiment terminated at 10:00 A.M., August 25. The stain had reached *D*, and the upper margin of the segment 1.5 cm. out from *E*. *A* was about 150 cm. above the cut end of the petiole.

3. The lamina was torn across from margin to midrib, and then severed from the midrib for a distance of 34 cm. upward, so that a loose flap hung downward, attached to the midrib only by the commissural bundles. The cut petiole was placed in a 0.75 per cent solution of eosin. After 2 hours 26 minutes the stain had reached 25 cm. below the last intact vein at the top of the flap, having moved this distance through the commissural tracheids, in addition to 220 cm. upward through petiole and midrib.

20. Margin

The young blade as it emerges from the pseudostem is margined by a delicate wing, white or tinged with red, but containing no chlorophyll, about 2.5 mm. broad. About 170 μ thick where it joins the marginal bundle, the wing narrows to a fine edge, and the outermost portion is scarious and transparent. It is composed entirely of thin-walled parenchyma, with an upper epidermis of small unthickened cells, and on the lower surface are individual cells containing mucilage, similar to those occurring in the epidermis of the precursory appendage. The marginal wing withers almost as soon as the leaf is exposed to the air, and dies off as far as the marginal vascular bundle.

Within the wing there occur a few small tracheids, followed by a very large tracheid about 125 μ in diameter, which occupies most of the cross-section of the lamina at this point, and a few somewhat smaller ones inside it. These elements must be regarded as forming a marginal bundle which is a separate entity, and not merely the longitudinal continuation of the veins, since the latter contain as they approach the margin no tracheids of this size. An almost continuous band, 1–1.5 mm. broad, of much smaller tracheids stretches inward from the large tracheids, and represents the marginal continuation of the veins. Very little phloem is present. The first mechanical tissue strengthening the margin occurs as narrow bands, 1–2 cells deep, of not very thick-walled fibers lying above and below

the bundles interior to the large marginal tracheids. Within this, strands of mechanical cells are present at intervals, and represent the distal extremities of the fibrous strands of the veins.

21. Splitting of lamina

The newly expanded leaf is entire. Older leaves are torn by the wind into narrow strips, parallel to the veins, which are usually distinct from one another as far as the pulvinar bands, and the tear may even proceed longitudinally along the midrib for a short distance (fig. 38). The leaf then appears to be pinnately compound, and the strips into which it is torn may well be called "false pinnae," since their physiological significance is the same as that of pinnae (compare the banana leaf in fig. 38 with the coconut leaves in the upper left corner of that figure).

The line of the tear made by the wind follows no course predetermined by the structure of the lamina, other than that it must run parallel to the veins; there are no anatomical differences which cause it to lie between one pair of veins rather than another. On the other hand, the resistances offered to the initiation or continuation of the tear are almost negligible. The marginal wing is short lived and is supplied with no mechanical elements; the marginal bundle contains a few weak mechanical strands which eventually bend parallel to the veins, and so present but a slight obstacle to the tear; the transverse septa include only isolated, inefficient thick-walled cells, and the commissural bundles are equipped with no mechanical elements to retard or stop its progress. Lippitsch (12) has made an interesting study of the development in several genera of the Scitamineae of those mechanical features which may retard the transverse tearing of the lamina. Many of these are better protected than *Musa sapientum*, and are still incapable of maintaining their integrity in the face of the wind. He believed the splitting to be autogenous, in that "primary tears" at right angles to the margin and penetrating the outer portion of the marginal bundle are initiated by the tension set up by the drying and contraction of the wing. These tears are continued inward as "secondary tears" by the action of external agents, of which the wind is the principal. Considering the ease with which the lamina may be split inward at any point, it

does not seem necessary to postulate any mechanism for the initiation of the tear to account for the laceration of the enormous lamina under the force of the breeze. According to PETERSEN (16) the cleft often begins in the body of the lamina and thence proceeds inward and outward. One certainly sees many incompleted splits

FIG. 38.—Old leaf, much frayed by wind, no longer able to support itself; such effete leaves wither in this position and drape the pseudostem.

of this character, but apparently they also are to be attributed to the shearing force of the wind. KARSTEN (quoted by GOEBEL 7) found that the margin of the lamina of *Heliconia dasyantha* dies before the central protion has ceased to enlarge. The dead margin persisting, tensions arise which result in the splitting of the lamina under the force of impact of rain drops, etc. GOEBEL (7) traces a suggestive

series in the compounding of monocotyledonous leaves. In the banana the false pinnae arise from the action of external forces on the mature leaf. In *Cyclanthus bipartitus* the time of the splitting of the two-lobed leaf is pushed forward, and occurs during its unfolding, resulting from tensions already present. In the palms the compounding of the leaf occurs still earlier, in the bud, where certain strips of tissue die or become slimy, isolating from each other the strips which make the pinnae.

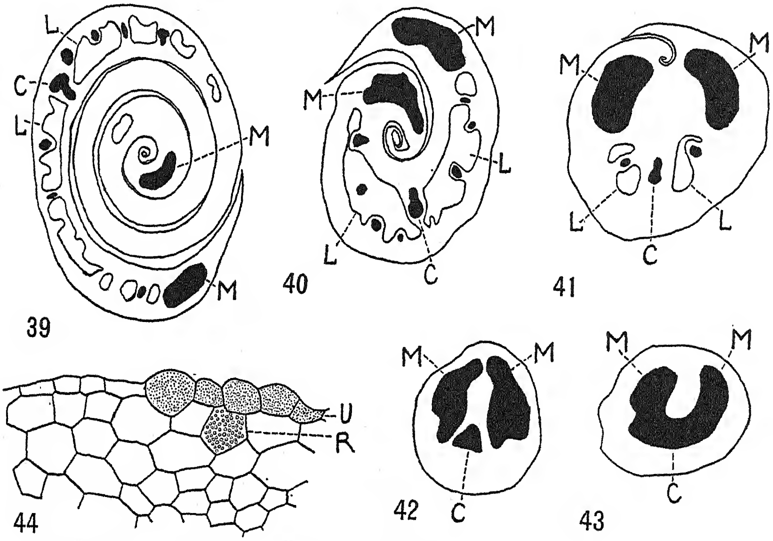
In the Gros Michel the behavior of the tissues adjacent to the tear is similar to that reported for *Musa ensete* by LIPPITSCH. The tissues die off as far as the second or third vein from the line of separation, and the pinna is bordered by a narrow (0.2–0.4 mm.) band of brown dead cells. The parenchyma surrounding this vein, especially on the side toward the wound, the water tissue, and the epidermal cells become thick-walled and suberized, the outer anticlinal walls often becoming considerably thickened. Often, however, the palisade cells between the first and second veins become suberized, at times in 5–6 vertical rows, as well as the water tissue and epidermis in continuity with them. At any rate, a complete wall of suberized tissue, often very irregular, closes off the wound. Fungal infections, however, sometimes spread inward from the tear as large yellowing areas. The wound left by the dying off of the margin is also closed off by suberized cells, so that the false pinna is protected on three sides by suberized membranes. Wound cork is never formed. LIPPITSCH points out that the tissue lost as a result of splitting represents a smaller outlay of material than would be necessary adequately to strengthen the leaf. He believes that this habit therefore conforms to the principle of conservation of material; and the lack of mechanical protection, inviting as it does the splitting, may be regarded as adaptive.

D. PRECURSORY APPENDAGE

22. STRUCTURE AND POSSIBLE FUNCTION

The cylinder formed by the convolute leaf narrows at its apex into the precursory appendage (figs. 9, 11), which in large leaves may reach 15 cm. in length. The coiled halves of the lamina continue upward as part of the appendage, which in its basal portion is

a helix in cross-section (figs. 39, 40). They gradually become narrower, and finally merge into the terete structure which is characteristic of the upper portion of the appendage (fig. 42). The veins at the apex of the lamina bend inward and upward very sharply, changing their course through as much as 135°, and joining the marginal bundle, with which they continue upward into the appendage. Because the appendage is so intimately connected with the blades



FIGS. 39–44.—Figs. 39–43, transverse sections of precursory appendage at various levels; ×12. Fig. 39, at base of appendage; fig. 40, 1 cm. above base; fig. 41, about 2.5 cm. above base; fig. 42, about 6 cm. above base; fig. 43, 7–8 cm. above base; *C*, central vascular bundle; *M*, marginal bundle; *L*, lacuna. Fig. 44, peripheral region of appendage in transverse section; *U*, mucilage cells; *R*, raphid bundle; ×135.

it must be torn off, along with the end of the right half, before the lamina can uncoil.

The appendage is a direct continuation of the midrib, and the bundles and lacunae of the latter extend upward into the base of the former (fig. 39 *C*, *L*). The lacunae contract and finally drop out, and the smaller bundles fuse and disappear. At about one-third the length of the appendage above the base only three bundles remain, the central one representing the continuation of the bundles of the midrib (fig. 42 *C*), and the other two the marginal bundles of the lamina (fig. 42 *M*). Already at the base of the appendage the mar-

ginal bundles were the most prominent, and as they continue upward their cross-sectional area relative to that of the entire appendage increases, until they occupy a large proportion of the cross-section of the organ. Finally the three bundles fuse and become indistinguishable. This was observed to happen in two ways. The marginal bundles may remain distinct from each other and fuse with the central bundle at their inner edges, forming an open ring (fig. 43), or they may first fuse with each other by their outer edges, and later with the central bundle at their inner edges, forming a closed ring. The single bundle present in the upper quarter of the organ becomes solid. The large marginal bundles are composed almost entirely of fairly wide tracheids, with a small amount of phloem scattered among them, and a larger portion of it at the inner edge of each bundle. In the predominance of xylem over phloem, these bundles resemble the marginal bundles of the lamina. Mechanical elements are absent from the appendage.

The ground tissue of the appendage is a parenchyma composed of thin-walled cells only slightly longer than broad. Cells containing raphid bundles are very frequent. The epidermis contains numerous stomata with large substomatal chambers, and large cells, elongated in the direction of the long axis of the appendage, which contain a deeply staining mucilage, and project above the general surface of the organ. These occur as isolated cells or are continuous in large groups (fig. 44). In addition, there are present epidermal cells of the usual type. The appendage never contains chlorophyll, and is white so long as it is still tightly inclosed in the pseudostem. When it emerges from the top of the latter it has already begun to turn brown, as a result of the withering and discoloration of the epidermal cells. Long before the lamina has completely emerged the appendage is black and shriveled, although it usually remains attached until it is torn off by the expansion of the lamina. The end curls up in drying, but the organ never assumes the corkscrew form described for other species.

Whatever may be the physiological function of the appendage, its mechanical significance seems clear.[9] It serves to maintain the

[9] For an interesting view on the morphological nature of the appendage, which space does not allow me to discuss here, see A. Arber, On the leaf tips of certain monocotyledons. Linn. Soc. Jour. Bot. 45 : 467–476, and the literature there cited.

young leaf in its coiled condition, and to prevent its fouling against the sheath which surrounds it during its long progress upward through the pseudostem. If the lamina ended abruptly, the folding back of its ends through friction against the sheath and the consequent entanglement of the leaf would be possible. By its tapering form the appendage is well adapted to push its way through the close-packed organs of the pseudostem, and to make a passage for the lamina which follows. This passage may be further aided by the lubricous surface created by the mucilaginous epidermal cells. The conclusion of GOEBEL (6) that the appendage is an *Abschlusskörper* seems a sound one.

The physiological function, if any, of the appendage in the Musaceae is not so clear. RACIBORSKI (17), whose studies were devoted principally to lianas, found that the stomata, vessels, intercellular spaces, and chlorophyll apparatus of the appendage mature earlier than in the body of the leaf, and that it transpires and assimilates before the latter. He observed that the earlier maturation of tissue is also true in the banana, although here photosynthesis is out of the question. Lianas, with their retarded leaf development, present conditions peculiar to themselves, and quite foreign to the banana. GENTER (5) suggested that the appendage is of service in closing off the central cavity of the pseudostem and preventing the access of rainwater and foreign matter to the young leaf. He also observed that if the appendage is freed from the surrounding sheaths and placed in a moist atmosphere, it guttates through the stomata. However, there is no experimental evidence that the organ has any non-mechanical physiological value to the plant.

In conclusion, it may be well to point again to the large number of scars which the leaf of the banana receives during the usual course of its development, both from the dying off of temporary organs and the imperfect adjustment between its parts. The withering of the margin and the precursory appendage, the tearing off of the end of the right side, all leave extensive scars which surround the lamina. These changes would occur in the most sheltered greenhouse as well as in the field, and perhaps it is not stretching the point too far to include the withered margins of the false pinnae in the same category, since the leaf is not adjusted to its habitat until

it has become falsely pinnate, and probably no large leaf on the plantation escapes being torn. If we turn to the internal tissues, the complete destruction of the protoxylem and the rhexigenetic origin of the lacunae should be included. The duration and fate of temporary organs during organogeny and development have received more attention from zoologists than from botanists, probably because plants are not so strongly individualized as animals. But the leaf of the banana is a highly individualized unit, which shows in a striking way the phenomenon of the abscission of temporary parts.

The investigations reported in the present article were made entirely on plants of the Gros Michel variety growing at Platfield, a plantation of the United Fruit Company near Richmond, Parish of St. Mary, Jamaica, where the writer spent a portion of the summer of 1926 as a guest of the Company. The work was completed in the botanical laboratory of the Johns Hopkins University. The writer desires to express his indebtedness to Dr. John R. Johnston, Director of Agricultural Research, for suggesting these investigations and to him and the other officials of the United Fruit Company, for making it possible to carry them out; to Professor Duncan S. Johnson, Johns Hopkins University, for many helpful suggestions, and to the superintendent at Platfield, Mr. T. D. Kieffer, and Mrs. Kieffer, for the innumerable kindnesses which made his sojourn on the plantation enjoyable as well as scientifically profitable.

Summary

1. The aerial shoot of the banana is leafy almost to the top of the pseudostem, and bears the largest leaves produced by the plant.

2. The phyllotaxy changes with the age of the plant, from under 2/5 to 4/9.

3. The lateral buds are not axillary, but arise *opposite* the axils.

4. The unrolling of the convolute leaf is accomplished only after the tearing away of a portion of the lamina.

5. Stomata occur on all the inclosed portions of the sheaths and stem.

6. The hypodermal layer of the outer surface of the sheath be-

comes first suberized, then lignified, as the sheath is pushed toward the exterior of the pseudostem.

7. The protoxylem elements of sheath, midrib, and principal veins are disrupted and their lumina occluded by parenchyma cells.

8. The lower surface of midrib and petiole is strengthened by two hypodermal strata of lignified cells.

9. A peculiar mode of anchoring the cuticle over the pulvinar bands is described.

10. Longitudinal furrows in the lamina are caused by pressure in the bud.

11. There is considerable variation in the density of stomata in different portions of the same surface of the lamina.

12. There is a marked increase in the number of veins near the margin over that near the midrib.

13. The efficiency of the commissural bundles in water transport was clearly demonstrated by experiment.

14. The wounds caused by the splitting of the lamina, and the dying off of the margin, are closed by the suberization of pre-existing cells.

15. The marginal bundles of the lamina become the most important vascular supply of the precursory appendage.

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CYTOLOGICAL STUDIES IN THE GENUS TYPHA[1]

MURIEL V. ROSCOE

(WITH PLATES XI, XII AND FOURTEEN FIGURES)

In a report on the flora of the Boston district (13), there is appended to the notation of the presence of *Typha latifolia* L. and *T. angustifolia* L. the following: "Where the two species grow together there are all kinds of intermediate forms. This is especially noticeable in the big swamps at West Cambridge."

This tendency to develop intermediate forms has been found to be by no means limited to West Cambridge, but is general in localities where the two species grow in any proximity to each other; thus forms grading into the typical *latifolia* and *angustifolia* are common. Further, it is known that hybrids of these two species occur in northern Europe.

This investigation was undertaken to ascertain the chromosomal conditions in the parent species and the suspected hybrids. Also, before the close of the investigation, some material from New Zealand, *T. angustifolia* L. var. *muelleri* Graeb. was received. Since *T. latifolia* is absent from New Zealand, the material was examined to note any resemblances and differences between this New Zealand variety and the *T. angustifolia* of New England.

Materials and methods

Material of both *T. angustifolia* and *T. latifolia*, as well as of forms lying systematically somewhere between the two, was obtained growing in various localities in Massachusetts. *T. latifolia* was also gathered in various places in Nova Scotia; while I am indebted to Professor JEFFREY for material of *T. angustifolia* var. *muelleri* from New Zealand.

Chromo-acetic (0.75 per cent) solution was used as a fixative except in the case of the New Zealand material, which was preserved in Carnoy's fluid. After the anthers were cut with a sharp razor, the

[1] Contribution from the Laboratories of Plant Morphology, Harvard University.

material was placed immediately in the fixative and pumped. Collecting was confined to the warmer parts of fine, warm days.

Following imbedding in nitrocellulose, the buds were cut at thicknesses of 5 and 10 μ, and stained with Haidenhain's iron-alum haematoxylin. For the study of division stages no counter-stain was employed, while for pollen study safranin was used as a counter-stain after haematoxylin. The sections were studied with the assistance of a 1.5 mm. Zeiss apochromatic objective and a no. 12 compensating ocular.

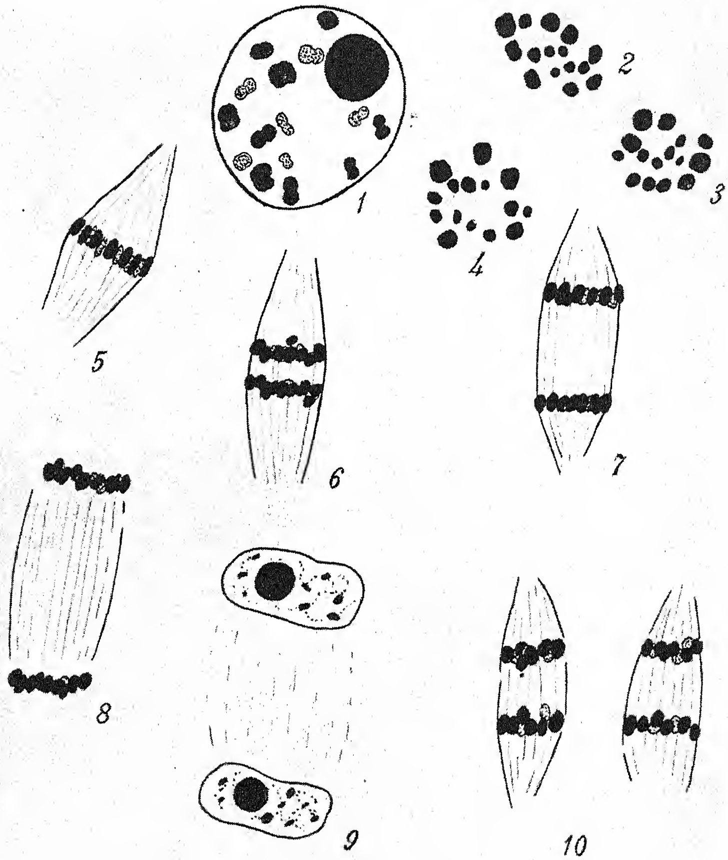
Observations

1. T. LATIFOLIA.—The divisions of *T. latifolia* are illustrated in figs. 1–10, and were contained in material collected from one pond margin in Nova Scotia.

The difficulties presented by diakinesis were great, and were due not only to the presence of a large nucleolus combined with the small size of the chromatin elements, but also to the intimate union of the latter in bivalent formation. In the most favorable cases, the situation is as shown in fig. 1, where all of the chromatin units seem double, and diakinesis may be settled as revealing fifteen bivalent chromosomes, which vary considerably in size. It is generally true that three of the bivalents in diakinesis are larger than the others (fig. 1), and a variation in the size of the remainder can be noted. Polar views of metaphase plates are definite in establishing the chromosome number as fifteen, and also show that the different sizes suggested by diakinesis are actually existent. It has not, however, been considered possible to arrange the chromosomes in any absolute categories, for the number of large and of small elements varies. Withal, the size difference of the chromosomes is dependent to some degree upon their plane of orientation on the spindle. The variations in size are thus most conspicuous in polar view of metaphase, and are usually not discernible in profile views of the plates or in subsequent stages. Also some preparations show a greater size difference than others, suggesting that the relative size is not an invariable quantity.

There is nothing extraordinary featuring the general course of meiosis, and heterotypic division (figs. 2–9) is completed with the formation of the two daughter nuclei, which are organized for the

resting period previous to the second division. In this, as in the first division, nothing abnormal or irregular is noted. A representative



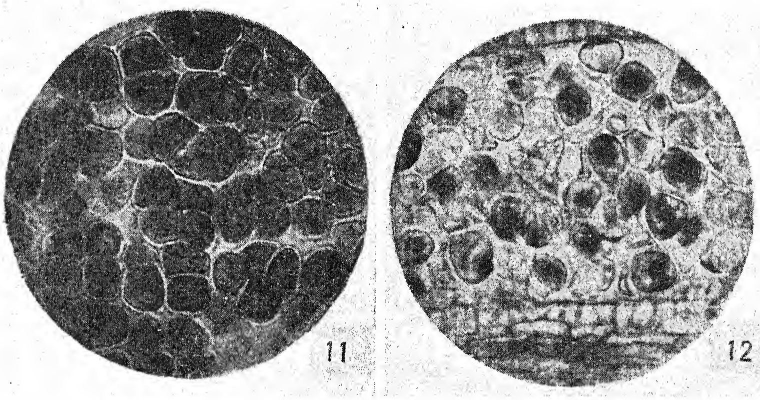
FIGS. 1–10.—*T. latifolia:* fig. 1, diakinesis; figs. 2–4, heterotypic metaphase, polar view; fig. 5, heterotypic metaphase, profile view; figs. 6–8, heterotypic anaphase; fig. 9, interkinesis; fig. 10, homotypic anaphase; ×approximately 2600.

anaphase is shown in fig. 10. At the conclusion of the homotypic, the normal tetrad of four similar microspores is developed.

It is significant that the pollen which is produced after such divisions is morphologically perfect. All the grains reach maturity,

and, when shed, retain the peculiar tetrad formation which is characteristic of *T. latifolia* (fig. 11). A study of pollen of this species when growing removed from *T. angustifolia* has shown it in all cases to consist of perfect grains.

In the establishment of these conclusions, some hundreds of pollen mother cells in division have passed under observation, and all stages submitted to repeated verification; thus cells showing metaphase plates, polar view, have been counted in upward of a hundred cells.



FIGS. 11, 12.—Pollen: fig. 11, *T. latifolia;* fig. 12, *T. angustifolia;* ×approximately 600

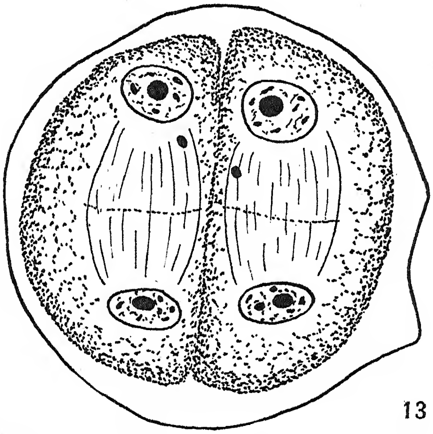
It may further be noted that some proportion of sterile pollen is frequently found in material gathered from what appears to be "pure" *T. latifolia* growing in New England, but such a pollen condition has never been found in any *latifolia* pollen from plants isolated as they are in Nova Scotia.

2. T. ANGUSTIFOLIA HYBRID.—This form approached *T. angustifolia* in appearance, but had a somewhat broader leaf and shorter interval than is usually found in the typical species. In such plants prophase usually discloses fifteen units. Metaphases may be regular and show only bivalents, but frequently metaphases with both bivalents and univalents are found. In such cases it is possible that a very loose union exists and some of the homologous chromosomes are early dissociated. Anaphases both regular and irregular have been observed, but all the chromosomes become included at inter-

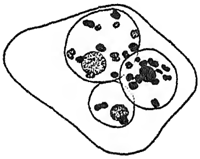
kinesis. At the close of the homotypic division, however, some of the chromosomes are occasionally left out of the daughter nuclei (fig. 13).

A somewhat unusual pollen mother cell is shown in fig. 14, where three nuclei of as many sizes are present, and contain varying numbers of chromatin elements.

3. T. ANGUSTIFOLIA.—Plate XI shows meiotic divisions for *T. angustifolia*. Fig. 15 represents an early prophase, in which some chromatin-like material is in actual passage from one cell to another.



13



14

FIGS. 13, 14.—*T. angustifolia* hybrid: fig. 13, homotypic telophase; ×approximately 2600; fig. 14, abnormal pollen mother cell with three nuclei; ×approximately 700.

This material seems to be intimately connected with the chromatin masses within the nuclei, one of which is becoming involved in the transmission. A number of cases showing black-staining material of this sort have been seen in *Typha;* such phenomena are conspicuous, but from the lack of a detailed study, no attempt is made to explain them.

Diakinesis is variable in regard to the number of chromosomes which appear. The same difficulty experienced in understanding this phase for *T. latifolia* is met here, but is enhanced in this case by the dissimilarity of different nuclei. In the one illustrated (fig. 16), eighteen elements may be counted, of which it is believed that all except the very smallest are bivalents; that is, we have twelve bivalents and six univalents. However, as many as twenty-two units

have been counted in other nuclei. This fluctuation in number may be explained as due to: (1) the disharmony of the parental chromosomes resulting in loose union; or (2) the discrepancy in numbers of the parental chromosomes. It is not yet possible to decide which of the two factors is in operation. The difficulty is increased because no chance to check by metaphase plates is presented, on account of the lagging of chromosomes on the spindle.

The manner in which the bivalents and univalents conduct themselves after the formation of the spindle varies from the course noted for *T. latifolia*. Figs. 23 and 24 are representative of early metaphase, and it will at once be noted that the departure from the norm is extreme. Not only is there delay in coming to the plate, but the variation in size, form, and relation of the chromosomes is great. Fig. 24 thus shows bivalents (both large and small), apparent univalents, and two cases where univalents have aligned themselves to appear as elongated masses of chromatin. Fig. 23 is even more anomalous, and shows sixteen chromosomes, of which one lying in the plate region is very large, and approached in size only by one of the two at the upper pole. The situation is pronounced. The suggestion might be made that these are two persistent nucleoli, but such does not seem to be a valid explanation, inasmuch as more than one nucleolus has never been observed in prophases, and also the prophase nucleolus is much larger (fig. 16). It is possible, if not probable, that the two masses are spherical aggregations of chromatin.

Later metaphases (figs. 19, 20) show that a larger number of the chromosomes reach the plate, but that always some univalents are left on the spindle. Fig. 20 is a rather rare condition, for usually more laggards appear than do in this figure. Consequently counts of polar view of such a stage are valueless. A large number of observations have shown fifteen chromosomes in such a "pseudo-plate" (fig. 17), but in two cases sixteen were realized, and one cannot be assured that the number is not still higher. From the fact of inconstancy in number in diakinesis, coupled with the variation in chromosome size, and the small size of all the elements concerned, as well as from the extreme irregularity of position assumed by the chromosomes on the spindle, it has been found impossible to settle upon a definite numerical value for the form under consideration.

Anaphase (figs. 18, 21) is usually irregular. Interkinesis frequently shows small masses of chromatin left outside the daughter nuclei, which may or may not form micro-nuclei.

One much elongated spindle with a peculiar disposition of the chromosomes was conspicuous (fig. 22). The spherical mass at the lower pole is comparable with similar masses noted in fig. 23, and is probably an abnormality resulting from the heterozygous character of the parent species.

A few homotypic divisions passed under review, and these showed some lagging, which, however, was not as pronounced as in the heterotypic. The end result is the formation of pollen which is crushed and sterile (fig. 12).

4. T. ANGUSTIFOLIA VAR. MUELLERI.—Plate XII shows stages in the division of pollen mother cells of material collected in New Zealand, and identified as *T. angustifolia* L. var. *muelleri* Graeb. by Dr. LEONARD COCKAYNE of Wellington, New Zealand.

Figs. 25–28 illustrate the heterotypic division, the figures of which are distinguished by regularity of conduct of the chromosomes. A feature of interest is presented by the number of chromosomes, which is thirty. This New Zealand variety of *T. angustifolia* is therefore a tetraploid form. Fig. 28 shows the thirty chromosomes at metaphase. There is a vast difference in the present instance between the general size of the elements, as contrasted with those in New England species of *Typha*, and it is apparent that the chromosomes are much smaller in the New Zealand variety. The homotypic division is likewise regular in the distribution of the chromosomes (fig. 29).

Discussion

1. CHROMOSOME NUMBERS.—The haploid chromosome number of fifteen, while not of any significance in itself, is a number seldom recorded for the higher plants.

2. NORMAL MEIOSIS.—Where meiosis is normal and is followed by the production of only fertile pollen, we have the expected condition for pure species. *Typha latifolia*, on the basis of these two features, together with its diploid chromosome number, is undoubtedly of uncontaminated origin.

3. ABNORMAL MEIOSIS.—Where meiosis is abnormal, and is followed by varying percentages of sterile pollen, the situation is linked

up with hybridity. This conclusion is warranted in view of the outstanding cytological contributions of recent years, which were inaugurated by ROSENBERG'S studies (15–17) on *Drosera*.

Usually irregularity of division is due to differences, either numerical or qualitative, in chromosome inheritance. Thus irregularities resulting from the hybridization of parents containing different numbers of chromosomes is seen in *Drosera obovata* ($2\times=30$), whose parents, *D. rotundifolia* and *D. longifolia*, contributed ten and twenty chromosomes respectively (17). COLLINS and MANN (8), however, decided from their researches on *Crepis* hybrids that chromosome dissimilarity and not chromosome number determines abnormal reduction. WINGE (19) expresses similar ideas when he says: "it is therefore certain that only such gametes as harmonize physiologically—or better, perhaps physiogenetically—can enter into the formation of a duality such as the sporophytic organism." He later concludes: "On the whole, imperfect reduction is associated with the hybrid nature." One thus feels justified in interpreting constantly occurring meiotic abnormalities as indicative of hybridism.

Viewed in such light, slight irregularities in *Typha* result when the parents are of nearly equivalent constitution, whereas greater abnormalities accrue when the parents are genetically dissimilar. The difference in the number of chromosomes noted in diakinesis and spindle figures of *T. angustifolia* may be due either to the difference in the numbers or to the physiological dissimilarity of the chromosomes contributed by the parents. In the latter case, the failure to form bivalents would increase the number of chromatin elements and explain the numbers recorded. At least, unlikeness of chromosomal units (either qualitative or numerical) leads to irregular divisions, and hence to the formation of gametes which either fail to reach maturity or else contribute an entirely different chromosome equipment to a new generation. In the latter case, unless an auto-regulative device is inaugurated whereby the chromosome number is reduced, these irregularities will be perpetuated.

4. TETRAPLOIDY.—The appearance of thirty haploid chromosomes in the New Zealand variety *muelleri* of *T. angustifolia* was a striking repetition of phenomena reported for many genera of plants. BLAKESLEE, BELLING, and FARNHAM (4) have limited the usage of

the term tetraploidy to the doubling of homologous chromosomes in a somatic cell, but the customary use of the term is to denote the condition of any species containing twice as many chromosomes as a closely related form. Since it is difficult to know whether or not chromosomes are homologous, if these elements are very small and manifest no recognizable morphological differences, the more general definition of the term seems preferable.

Perhaps the most outstanding case of tetraploidy in which the history is known is that described by Miss DIGBY (11) for *Primula kewensis*. The first appearance of this species was in 1899, when one aberrant individual appeared, obviously as a result of a natural cross between *P. floribunda* ($\times=9$) and *P. verticillata* ($\times=9$). In 1900 the same hybrid was obtained by artificially crossing the two supposed parents. The hybrid proved sterile, although, like its parents, it possessed the diploid number of chromosomes ($2\times=18$), and all the flowers were thrum-eyed. In 1905 one pin-eyed individual appeared, and, by fertilizing it with pollen from a thrum-eyed individual, a seedling was developed which contained eighteen haploid chromosomes and was fertile. *P. kewensis* is an instance of a tetraploid form originating in material known to be of hybrid constitution.

BREMER (5, 6, 7) has made an extensive study of *Saccharum*, including many species and species hybrids within the genus. He relates a haploid number of fifty-six for *S. spontaneum* and of forty for many different races of *S. officinarum*. He finds further that Kassoercane, which is probably a spontaneous hybrid of *S. officinarum* and *S. spontaneum*, has sixty-eight chromosomes, and concerning the origin of such a number, BREMER (6) relates the following:

> The diploid chromosome number of these hybrids, which approximately reaches 136, can be explained only by assuming that, when fertilisation with a male nucleus of *S. spontaneum* (glagah) occurs, the number of chromosomes in the egg cell of the sugar-cane (*S. officinarum*) doubles, so that not 40 but 80 sugar-cane chromosomes meet with 56 *glagah* chromosomes, with which they form the diploid number 136. After reduction, the haploid chromosome number 68 then results.

In a recent *Rosa* contribution, BLACKBURN and HARRISON (2) find *R. wilsoni* falls in line with Miss DIGBY'S *Primula kewensis* and

BREMER'S *Saccharum*, both of which followed hybridization. *R. wilsoni* has a somatic number of forty-two, and undoubtedly was derived from a cross between *R. pimpinellifolia* (balanced tetraploid) and "some *Tomentosa microgene*" (unbalanced pentaploids, whose microspores when functional carry seven chromosomes and the egg cells twenty-eight). "With *pimpinellifolia* as seed parent, the cross should have 14+7 (=21) in its somatic nuclei." Since *R. wilsoni* has a chromosome number of forty-two, "it has, therefore, like *Primula kewensis*, doubled its original complement. In doing so, again like that hybrid, it has attained fertility."

Species with twice as many chromosomes as related ones are reported in many genera: *Dahlia* (12), *Aegilops* (1), *Rosa* (18), and *Rubus* (14). DENHAM (9, 10) counted the chromosomes in thirty-two varieties of cotton, and found New World and Egyptian cottons were tetraploid ($\times=26$) and Asiatic cottons diploid ($\times=13$). No explanation is offered by him concerning the appearance of the larger number. The numbers given by WINGE for *Atriplex patulum* and for *Chenopodium bonus-henricus* illustrate chromosome doubling in the Chenopodiaceae. The list of such cases might be extended to cover most of the genera which contain large numbers of species, for tetraploid forms are common within polymorphic groups.

From a consideration of the known results of other workers, and from his own extensive observations, WINGE says the doubling of the chromosome number should be explained "not as a cleavage of the chromosomes, but as an indication of hybridization, and thus an addition of the chromosomes." Such a result is due to the coming together of gametes of too little harmony. BLACKBURN and HARRISON (3) find in the Salicaceae normally acting tetraploid species, both in *Salix* and *Populus*. They view hybridity as a spur to the duplication of chromosomes, and suggest that in general tetraploid species probably arose in one of two ways: (1) two nuclei become included in one cell; (2) the chromosomes become fragmented without subsequent cell division, an hypothesis advocated by WINGE. A third method seems justifiable where diakinesis reveals only unpaired chromosomes, due to complete disharmony of the parental gametes, a conclusion which follows from WINGE'S ideas.

There is a certain similarity between the chromosomal condition

of the previous cases and of *Typha angustifolia* var. *muelleri*. In plants like *Primula kewensis*, where the ancestry and cytological conditions are known, it is easier to postulate the origin of a doubled number, or at least to note that, regardless of the method of doubling, the tetraploid condition is connected with heterozygous ancestry. In the *Typha* under discussion, nothing is known of the parent stock, either historically or cytologically; but in comparison with *T. latifolia* of Eastern America, it is a form which has doubled its chromosome complement. It distributes its chromosomes equally in sets of thirty, and forms tetrads normally. The percentage of viable pollen, in the absence of mature grains, has not to date been estimated.

By analogy with the development of tetraploidy within the genera *Primula*, *Saccharum*, and *Rosa* in known hybrid stock, the possibility is offered that the chromosome number in *Typha angustifolia* var. *muelleri* has resulted from crossing. The parents involved are unknown and quite possibly are no longer existent.

Conclusions

Typha latifolia, when growing beyond the range of *T. angustifolia*, becomes geographically monotypic. A study of the pollen mother cells of this species shows fifteen haploid chromosomes and complete regularity of meiosis, which leads ultimately to an equal distribution of the chromosomes and the formation of only perfect pollen. The latter, when mature, is fully protoplasmic, contains both vegetative and generative nuclei, and is shed in the characteristic "tetrads" of the species.

T. latifolia, when growing in New England, may be "pure," and present cytological features agreeing exactly with those of material isolated from *T. angustifolia*. Close to this, however, are forms which show slightly irregular chromosomal action, and develop a proportion of sterile pollen.

In *T. angustifolia* the number of chromosomes has not been fixed as an invariable quantity, but a larger number appear than were present in *T. latifolia*. The increased number is explained by the presence of univalent chromosomes. These are caused either by disharmony of the parental chromosomes, giving a loose union in

diakinesis and early metaphase stages, or by dissimilarity of the chromosome numbers of the parents. In either case the phenomenon appears to result from hybridity.

Irregular meiotic figures are abundant in *T. angustifolia*, and the small or univalent chromosomes are usually responsible for the lagging. Ordinarily, the tardy action of the univalents leads to the exclusion of a few of these from the tetraspore nuclei. Further abnormality is revealed by the large masses of chromatin formed in some cases (figs. 22, 23), as also by the position of the chromosomes on the spindles. The pollen grains disclose a high degree of malformation and abortion, and at time of shedding only a small percentage show normally protoplasmic conditions.

Forms approaching *T. angustifolia* in taxonomic characters have been found abundant, which, when examined, disclose a large amount of pollen sterility and varying degrees of chromosome irregularity.

The conclusion is reached that *Typha latifolia* shows only the cytological characteristics of a "pure" species in the northern part of its range, but when in proximity to *T. angustifolia* tends to hybridize with it and produce both latifolioid and angustifolioid hybrids.

T. angustifolia seems never to reveal the regularity of meiosis achieved by *T. latifolia*, and develops only a comparatively small percentage of well-formed pollen. It appears probable that this species has arisen through crossing, and now contributes to the further formation of new intermediates through hybridizing with other forms.

T. angustifolia var. *muelleri* shows tetraploidy, and from a comparison with tetraploid species in other genera, such as *Primula*, *Saccharum*, etc., it is believed that this variety of *angustifolia* owes its doubled chromosome number to crossing, either in its remote or recent history.

Summary

1. The fundamental chromosome number of *Typha* is fifteen.

2. When *T. latifolia* grows beyond the range of *T. angustifolia*, the dividing pollen mother cells show normal chromosome distribution, and the pollen consists of uniformly protoplasmic grains.

3. The haploid chromosome number in *T. latifolia* is fifteen.

4. Where *T. latifolia* ranges with *T. angustifolia*, forms appear which show slightly irregular chromosome action and sterile pollen.

5. A larger number of chromosomes appears in *T. angustifolia* than in *T. latifolia;* this is due to the presence of univalents.

6. *T. angustifolia* shows irregularities in meiosis, which consist of lagging, univalent chromosomes, and the frequent exclusion of these from the daughter nuclei.

7. Mature pollen of *T. angustifolia* shows a high percentage of malformed and abortive pollen grains.

8. Intermediates between *T. latifolia* and *T. angustifolia* appear where these two species range together. These show varying degrees of meiotic irregularity, leading eventually to the formation of sterile pollen.

9. The conclusion is reached that *T. latifolia*, when removed from *T. angustifolia*, shows the cytological characteristics of a "pure" species. *T. angustifolia*, however, reveals abnormalities characteristic of hybrids, namely, irregular chromosome distribution and pollen sterility.

10. *T. angustifolia* var. *muelleri* contains thirty haploid chromosomes. Hybridization is offered as a possible explanation of this tetraploidy.

This investigation has been carried on under the supervision of Professor E. C. JEFFREY. I am indebted to him for his suggestion of the study, and for his interest and assistance during its progress.

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EXPLANATION OF PLATES XI, XII

Plate XI, *Typha angustifolia* L., pollen formation; ×approximately 2600.

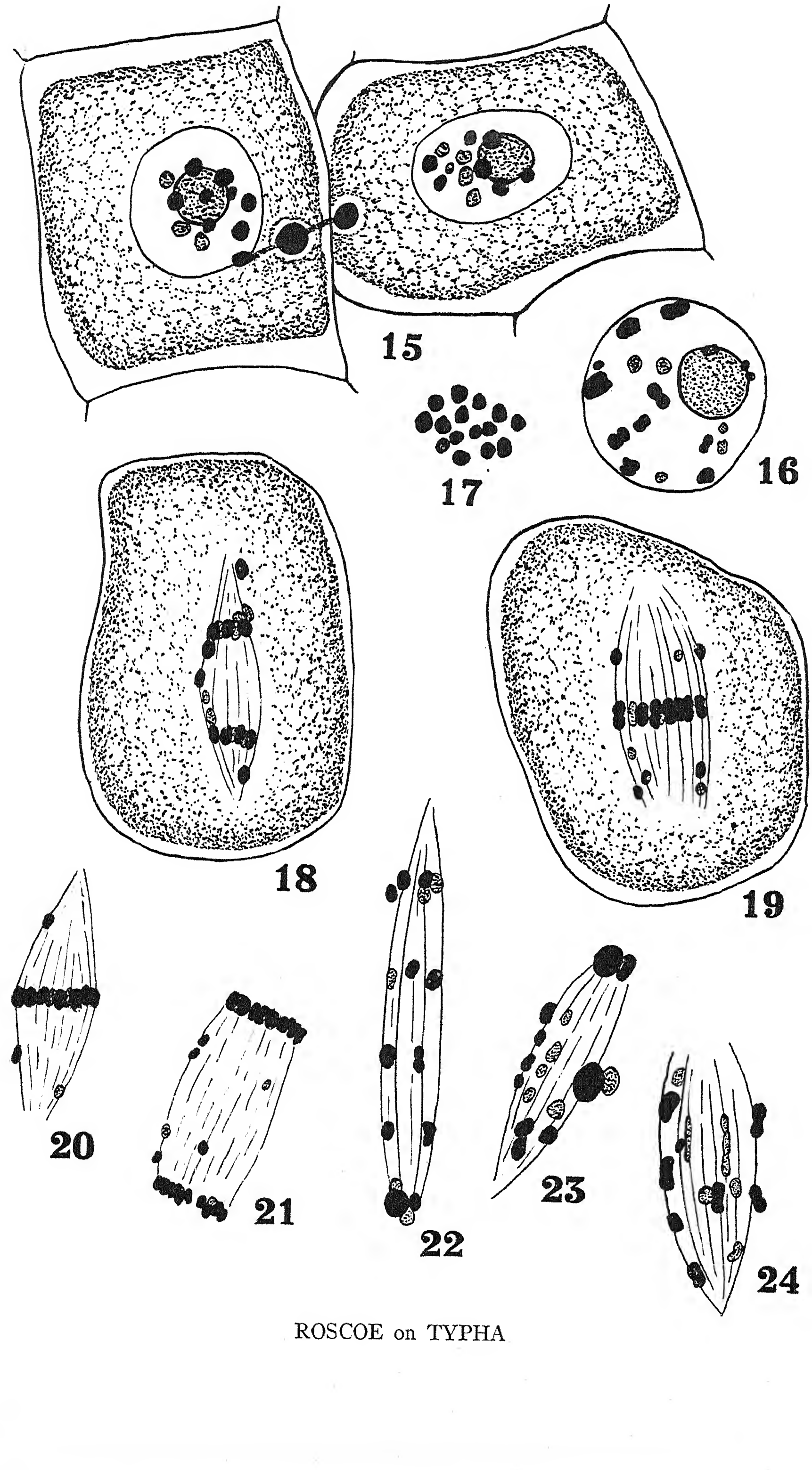
FIG. 15.—Early prophase, showing passage of chromatin-like material from one cell to another.

FIG. 16.—Diakinesis.

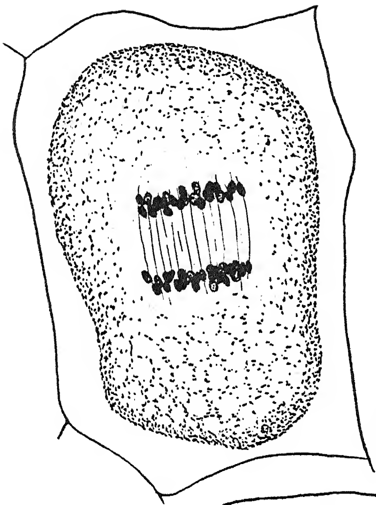
FIG. 17.—Heterotypic metaphase; polar view of a "pseudo-plate."
FIG. 18.—Heterotypic anaphase.
FIG. 19.—Heterotypic metaphase.
FIG. 20.—Heterotypic metaphase.
FIG. 21.—Late heterotypic anaphase.
FIG. 22.—Abnormal elongated spindle in heterotypic division.
FIG. 23.—Early heterotypic metaphase.
FIG. 24.—Early heterotypic metaphase.

Plate XII, *Typha angustifolia* L. var. *muelleri* Graeb., pollen formation; ×approximately 2600.

FIG. 25.—Heterotypic anaphase.
FIG. 26.—Heterotypic metaphase.
FIG. 27.—Late heterotypic anaphase.
FIG. 28.—Heterotypic metaphase, polar view.
FIG. 29.—Late homotypic anaphase.



ROSCOE on TYPHA



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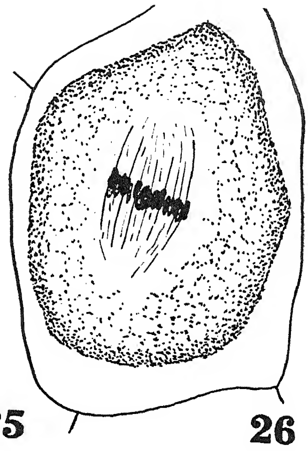

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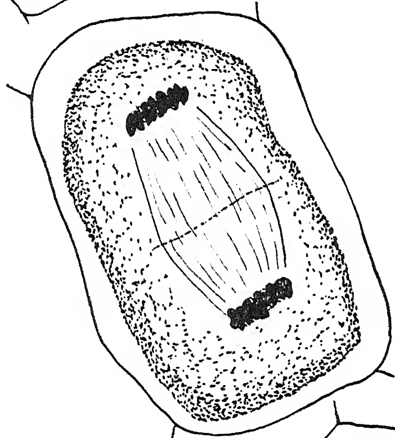

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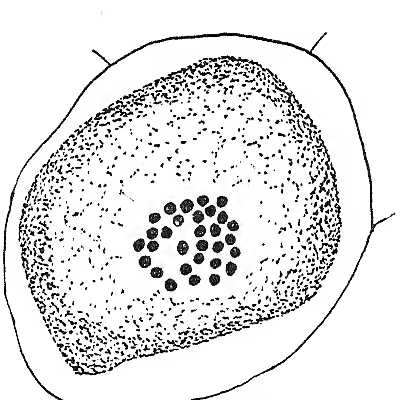

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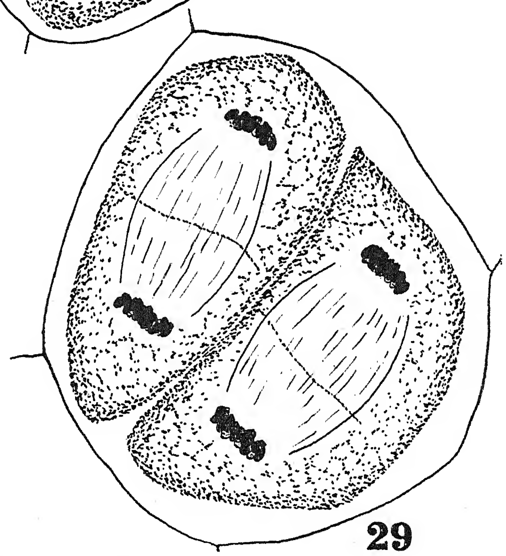

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ROSCOE on TYPHA

IRON REQUIREMENT FOR CHLORELLA

E. F. HOPKINS AND F. B. WANN[1]

Introduction

(WITH THREE FIGURES)

Although it has been recognized for many years that iron is necessary for the normal development of green plants, very few experimental data on the actual amounts of this element needed to support growth are available. The usual "trace" supplied in most nutrient solutions has always been deemed ample for the plant's needs, and in some cases iron as such is not even included in the formula for the culture solution, it being conceded that enough of the element is supplied as an impurity in the other salts. It is obvious that the amount of iron added to a particular type of nutrient solution as a "trace" will vary directly with the number of investigators employing this solution, while the amounts derived from the other salts as an impurity will depend on the source of the reagents used. Even with iron present in the solution and apparently available to the plant, it is conceivable that it may still be a limiting factor for growth.

Very little is known of the rôle of iron in plant nutrition, although it is generally regarded as being necessary for chlorophyll formation. Various types of chloroses lend support to this idea. Lime-induced chlorosis, extensively investigated by GILE and CARRERO (5) and others, appears to be due merely to a depression in the availability of iron in soils with high lime content and consequent low H-ion concentration. In soils of this type the iron is undoubtedly removed from solution both by precipitation and adsorption. In the case also of the manganiferous soils studied by JOHNSON (8), chlorosis results from the unavailability of iron. Although the reaction of these latter soils is such that ferrous iron would be available

[1] The completion of this joint project was made possible through the cooperation of the National Research Council and Cornell University, to both of which institutions the authors acknowledge their indebtedness.

for plants, this form of iron is readily oxidized by the manganese to the ferric condition, and then precipitated at the H-ion concentration normally characteristic of the soils in question. The relation of the H-ion concentration to the chlorosis of wheat plants has been studied by McCall and Haag (11), who found this condition arising in solutions of pH 4.02 to 7. They concluded that the iron was unavailable, or that a faulty metabolism resulted from the immobility of iron in the plant.

It has recently been suggested by Oddo and Pollacci (13) that iron is necessary for the formation of the pyrrole grouping in chlorophyll, the element in this case functioning as a catalyst. These investigators reported that they were able to obtain normal green plants in solutions supplied with the pyrrole compound but lacking iron. In an attempt to repeat this work, however, Deuber (4) failed to substantiate their results, but found on the contrary that pyrrole was quite toxic to the several plants used, and apparently could not be substituted for iron in the culture solution.

With reference to the algae, Benecke (2) states that iron salts act almost entirely as chemical stimulators, and doubts that the necessity of this element can be demonstrated with certainty with algal cultures. Since many of the unicellular green algae can be cultivated on organic media in the dark under pure culture conditions, it would appear that iron may function as a stimulator as in the case of the fungi. The necessity of iron for the latter plants is still an open question. Molisch (12) found that on iron-free solutions spore production was inhibited, although iron was apparently unnecessary for vegetative growth. In one of his experiments, however, he obtained practically no mycelium when the iron was completely removed by previous fungus growth. Currie (3) states that "iron is not at all necessary for the development of spores" in iron-free cultures of *Aspergillus niger*. The iron-free salts employed by Currie were prepared by recrystallization in platinum, but according to Stokes and Cain (14) all platinum contains a small amount of iron, and it may therefore be questioned whether Currie's solutions were absolutely iron-free. Algae grown in the dark still develop chlorophyll (1), however, and apparently require iron for growth. It would appear that iron plays a rôle entirely apart

from the functioning of chlorophyll in photosynthesis; and its possible connection with respiration, as suggested by WARBURG (17), seems to offer the most promising field for investigation in this connection.

Considerable attention has been directed in recent years to the source of the iron supply in nutrient solutions as affected by the H-ion concentration. GILE and CARRERO (5) studied the assimilation of iron by rice in acid, neutral, and alkaline culture solutions. Ferric chloride they found was practically unavailable in the alkaline cultures, whereas ferric citrate, in the lower concentration employed, yielded just as good growth in the alkaline as in the acid solution. The relation of the H-ion concentration to the availability of iron for *Chlorella* has been pointed out in a recent paper by the writers (7). The unavailability of iron in nutrient solutions at the higher pH values was discussed in connection with the results presented in that paper.

The difficulties involved in obtaining accurate data on the actual amounts of iron needed by the higher green plants is of course evident. The iron content of the seed is always a variable factor, and it would appear to be practically impossible to start with seedlings of no iron content. Securing absolutely iron-free solutions is also difficult. Nevertheless JONES and SHIVE (9) have shown a fairly consistent increase in dry weight of crop with increase in iron content of the solution, indicating that iron may possibly be a limiting factor. The small number of plants used and lack of consideration of the H-ion concentration of the solutions, however, prevent much weight being given their conclusions. In the solutions of very low iron content the plants were chlorotic. In a later paper (10) they have shown that in certain solutions, such as Tottingham's, low yields and chlorosis are associated with high pH values when ferric phosphate is used as a source of iron. This form of iron is available, however, when the solutions contain ammonium sulphate. An explanation of this may perhaps be found in our experiments already mentioned, in which it was shown that a greater absorption of the ammonium-ion from ammonium salts increased the H-ion concentration of the culture solutions. This would tend to make the iron soluble. In general, JONES and SHIVE find that the nature of the

solution with respect to the salt constituents and H-ion concentration appears to determine the availability and efficiency of a given iron salt for plant growth.

In some of their experiments GILE and CARRERO estimate that the amount of available iron must have been less than one part in 10 million of solution. They also obtained larger yields by increasing the amount of iron added to the solutions. Apparently rice was able to assimilate only soluble iron, colloidal iron being unavailable. The necessity of determining the exact amount of soluble iron present in the culture solutions is apparent.

The use of pure cultures of unicellular green algae offers certain distinct advantages in problems of this character. For ease of manipulation, volume of solution required, and exactness of crop determination they are easily superior to higher plants. Moreover, the small amount of inoculum necessary permits of practically complete elimination of any element under study, provided this element can be eliminated from the culture solution. Individual variation is entirely absent where inoculations are made from a uniform suspension of cells from a single stock culture.

In some of our earlier experiments (7) on the effect of the H-ion concentration on the availability of iron for the unicellular green alga *Chlorella*, it was observed that in buffered nutrient solutions containing calcium at pH 6 and above, a precipitate of calcium phosphate was produced on which the iron added to the solution was completely adsorbed, even after the addition of sodium citrate. The alga failed to grow in such solutions. The property of adsorption possessed by the calcium phosphate precipitate suggested a method of obtaining a nutrient solution completely free from iron, whereby the necessity of this element for growth, or the actual amounts needed to permit growth, could be studied.

Methods

BASIC IRON-FREE CULTURE SOLUTION.—This solution contained in addition to glucose all the essential mineral elements except iron. Any iron present as an impurity in the compounds used was removed by adsorption on the calcium phosphate precipitate formed

in the preparation of the solution. The following technique was employed.

Two separate lots of one liter each of a solution, designated solution A, were prepared in pyrex flasks of 2-liter capacity. Similar portions of solution B were made up in 3-liter pyrex flasks. The composition of the solutions was as follows:

Solution A		Solution B	
$Ca(NO_3)_2.4H_2O$	5.9 gm.	K_2HPO_4	46.46 gm.
$MgSO_4.7H_2O$	0.8 gm.	Distilled water to	1000 cc.
Glucose	40.0 gm.		
Distilled water to	1000 cc.		

The solutions were sterilized, and when cool one lot of solution A was poured into each of the flasks containing solution B. A heavy precipitate was produced in both mixtures. The mixed solutions were allowed to stand several days, being shaken up a number of times during this interval. The precipitate was then permitted to settle out completely, leaving a clear supernatant liquid. This part of the solution was removed from each of the mixtures by means of a sterile siphon of pyrex glass, the two portions being combined in a single larger sterile pyrex flask. The basic culture solution thus obtained was sterile, was presumably free from iron, and had come in contact with nothing but pyrex glass in its preparation. A test on a portion of the solution showed no iron present.

Standard iron solution.—A standard iron solution was prepared by dissolving 100 mg. of pure iron wire in 5 cc. concentrated HNO_3 to which a little water was added. This solution was evaporated to dryness and the iron taken up in dilute redistilled HCl. This was again evaporated to dryness and the iron finally dissolved in 10 cc. dilute HCl and made up to volume in a 200 cc. volumetric flask. One cc. of this solution contained 0.5 mg. Fe.

Addition of sodium citrate.—As the experiment was originally outlined, it was planned to introduce known amounts of iron (ranging from 0 to 5 mg. Fe per culture) to the various culture flasks, add an equal portion of sodium citrate solution to each, and make up the volume to 25 cc. in all. To these solutions of differing iron con-

tent 25 cc. of the basic iron-free nutrient solution was to be added, making the complete nutrient solution of 50 cc. for each culture.

The use of sodium citrate in conjunction with the standard iron solutions introduced a possible source of iron contamination. Several samples of the salt were tested for iron, and all contained considerable quantities; hence the addition of the salt to the standard iron solutions would introduce an error in the actual amounts of iron added, and the check cultures, receiving sodium citrate but no iron, would in reality receive iron as an impurity in the citrate.

To overcome this difficulty, the possibility of adding the sodium citrate to solution A in order to remove the iron from this salt by adsorption on the calcium phosphate precipitate was investigated. It was found, however, that the citrate was apparently carried down with the precipitate to such an extent that the resulting clear liquid would not subsequently hold iron in solution at pH 7.5. In this connection it was also determined that iron adsorbed by the calcium phosphate precipitate could not readily be brought back into solution by the addition of more sodium citrate.

The only alternative was to obtain sodium citrate free from iron. This was done by recrystallization. A considerable quantity of C.P. salt ($2Na_3C_6H_5O_7 \cdot 11H_2O$) was recrystallized twice from distilled water, and finally from conductivity water in pyrex glass vessels. The resulting salt consisted of very minute crystals and was practically free from iron. A portion of the salt dried at 90° C. showed 78 per cent sodium citrate.

In previous experiments it was found that 0.01 gm. of sodium citrate was sufficient to hold 0.05 mg. of iron in solution in each 50 cc. culture. In the present series, however, the use of larger amounts of iron was planned, and it was also realized that some calcium would be present in the basic iron-free nutrient solution which might affect the solubility of iron. To test the ability of the recrystallized sodium citrate to hold 5.0 mg. of Fe in solution in the complete culture medium, the following trial was made. To 10 cc. of standard iron solution (5 mg. Fe) 0.5 cc. of a 2 per cent sodium citrate solution (0.01 gm. Na citrate) was added in a small Erlenmeyer flask. Solutions A and B were prepared and mixed, the usual precipitate of calcium phosphate forming. The clear iron-free solu-

tion was obtained by centrifuging and adding 50 cc. to the iron-sodium citrate solution. A precipitate resulted. It was evident that the iron was thrown out of solution. Further tests were then made with increasing amounts of citrate added to 10 cc. of standard iron solution. In each case the volume was brought up to 20 cc. with water, and 50 cc. of the iron-free nutrient solution then added. It was found that 1.5 or 2 cc. of the 2 per cent sodium citrate solution would prevent the precipitation of 5 mg. Fe under these conditions. Thus by using 0.04 gm. sodium citrate per culture, it was possible to hold the largest amount of iron added (5 mg.) completely in solution with all the other salts of the complete nutrient solution present and at a pH of approximately 7.5.

PREPARATION OF SOLUTIONS OF DIFFERING IRON CONTENT.—About 75 pyrex Erlenmeyer flasks of 150 cc. capacity were thoroughly cleaned and drained. To each of these (except for solutions nos. 1 and 2) 1 cc. of a 4 per cent citrate solution (0.04 gm.) was added, using a pipette of pyrex glass. Iron was then added to these in groups of four for each concentration, as shown in table I. The total volume was brought up to 25 cc. in each of the culture flasks by the addition of the necessary amount of redistilled water, as indicated in the table, allowing for 1 cc. of sodium citrate solution in all cultures except with solution nos. 1 and 2, in which cultures it was proposed to omit both iron and sodium citrate. The culture flasks were then sterilized at 15 lb. for 30 minutes.

The sterile basic iron-free culture solution was then added to the culture flasks in 25 cc. quantities by means of sterile pipettes. The final volume of each complete culture solution was thus 50 cc. The original concentrations of solutions A and B, as presented earlier, were reduced to one-fourth these values by the two combinations involved in the preparation of the final culture medium.

Three flasks of each different concentration of iron, three "blanks" in pyrex flasks, and three "blanks" in quartz flasks (no citrate and no iron) were then inoculated with a suspension of *Chlorella* cells. The date of inoculation was January 5, 1926. The suspension was prepared in a portion of the sterile iron-free solution, a pure culture of the organism on mineral nutrient agar supplying the cells. The only iron introduced with the suspension was that

present in the cells themselves. Each inoculated culture received 0.5 cc. of this suspension. The remaining uninoculated flasks served for initial iron and pH determinations.

Conductivity water in pyrex flasks showed no iron after long sterilization, so that the possibility of iron dissolving from the pyrex glass is remote. With the exception of some of the pipettes, all glassware used was of pyrex.

TABLE I

COMPOSITION AND INITIAL IRON CONTENT OF NUTRIENT SOLUTIONS; EXPERIMENT 1

SOLUTION NO.	CUBIC CENTIMETERS OF IRON STANDARD NO.			REDISTILLED H_2O (CC.)	4% NA CITRATE SOLUTION (CC.)	BASIC FE-FREE SOLUTION (CC.)	MG. FE PER CULTURE	
	1	2*	3†				Added	Found
1.....	0.0	0.0	0.0	25.0	0	25	0.0000	Blank‡
2.....	0.0	0.0	0.0	25.0	0	"	0.0000	Blank
3.....	0.0	0.0	0.0	24.0	1	"	0.0000	Blank
4.....	0.0	0.0	0.2	23.8	1	"	0.0001	.....§
5.....	0.0	0.0	0.4	23.6	1	"	0.0002	———
6.....	0.0	0.0	0.8	23.2	1	"	0.0004	———
7.....	0.0	0.0	1.0	23.0	1	"	0.0005	———
8.....	0.0	0.0	2.0	22.0	1	"	0.0010	0.001‡
9.....	0.0	0.0	4.0	20.0	1	"	0.0020	0.0015
10.....	0.0	1.0	0.0	23.0	1	"	0.0050	0.0065
11.....	0.0	1.4	0.0	22.6	1	"	0.0070	0.0075
12.....	0.0	2.0	0.0	22.0	1	"	0.0100	0.01
13.....	0.0	4.0	0.0	20.0	1	"	0.0200	0.02
14.....	0.0	6.0	0.0	18.0	1	"	0.0300	0.03
15.....	0.0	10.0	0.0	14.0	1	"	0.0500	0.05
16.....	0.2	0.0	0.0	23.8	1	"	0.1000	0.1‡
17.....	1.0	0.0	0.0	23.0	1	"	1.5000	0.5‡
18.....	2.0	0.0	0.0	22.0	1	"	1.0000	1.0
19.....	10.0	0.0	0.0	14.0	1	"	5.0000	5.0

* 10 cc. of iron standard no. 1 diluted to 1000 cc. = 0.005 mg. Fe per cc.

† 1 cc. of iron standard no. 1 diluted to 1000 cc. = 0.0005 mg. Fe per cc.

‡ In fused quartz glassware; all other cultures in pyrex flasks.

§ The amounts of iron in the 10 cc. samples of solutions 4 to 7 inclusive were below the limit of the colorimetric test employed.

DETERMINATION OF INITIAL IRON CONTENT OF CULTURE SOLUTIONS.—Standard iron solutions were prepared in 50 cc. Nessler comparison tubes, using the method previously described (7). HCl redistilled through a pyrex condenser and measured with a pyrex pipette was employed. Fresh KSCN solution was used. The original iron wire solution was used for the more concentrated iron standards, and fresh dilutions of this stock were prepared for the lower iron concentrations. This is necessary because in dilute solution the iron

is apparently adsorbed on the walls of the volumetric flasks after standing for some time, and such solutions do not give true values for iron.

A blank determination on the reagents (HCl, KSCN, H_2O, and acetone) showed a trace of color when compared with a tube of water. The standard containing 0.0002 mg. Fe, however, could be distinguished from the reagent blank with certainty, although this appears to be the lower limit of the test. Differences between standards containing 0.0002 and 0.0003 mg. Fe were readily distinguished.

Ten cc. from each of the uninoculated culture solutions were now tested for iron by comparison with the preceding standards. These results are included in table I. It will be noted that the amounts of iron found are in close agreement with the calculated amounts added.

At the same time determinations of the initial H-ion concentration of the various solutions were made, using the GILLESPIE colorimetric method (6). Except for the solution containing 5 mg. Fe, the pH values were quite uniform throughout. This one exception can be accounted for by the addition of more free HCl with standard iron solution.

OBSERVATIONS ON GROWTH OF CULTURES.—One week after inoculation only a trace of growth was observed, this being confined to the cultures containing the higher concentrations of iron. This limited growth of the alga was conceivably due to the unavailability of nitrogen, as proposed in connection with Experiment 6 in the series of studies on the effect of pH on the growth of *Chlorella* (7). It was thought that the addition of NH_4NO_3 to the cultures might permit of more rapid growth. Accordingly on January 12, 1926, 1 cc. of sterile NH_4NO_3 solution containing 0.025 gm. per cc. was added to one culture of each iron concentration. The remaining cultures were not changed. It will be seen from the dry weights of the crops, however, that the addition of NH_4NO_3 was apparently without any effect on the growth of the organism.

Results of first experiment

On January 30 the following observations were made. Good growth was present in cultures with 5 and 1 mg. Fe, the color in

both lots being dark green. A rather copious flocculent precipitate was present in the first mentioned lot. At 0.5 mg. Fe there was very fair growth, but light green in color; at 0.1 mg. Fe, fair growth but brownish in color; at 0.05 mg. Fe, growth slight and color yellowish; at 0.03 and 0.02 mg. Fe, a trace of growth. In the remaining cultures there was no growth.

At this time the algal crops were harvested and the dry weights determined in the manner described in our earlier papers. Final pH

TABLE II

GROWTH OF CHLORELLA IN NUTRIENT SOLUTIONS WITH DIFFERING IRON CONCENTRATIONS; EXPERIMENT 1

Solution no.	PH of solution: Initial control	PH of solution: Final A	Final B	Final C	Fe in mg. per culture: Initial control	Fe in mg. per culture: Final A	Final B	Final C	Dry weight of crop (mg.): A	B	C	Average crop (mg.)
8*...	7.1	.....	.....	.....	0.001+	....	....	....	.....	.....	.....	.....
9...	7.1	.....	.....	.....	0.0015	....	....	....	.....	.....	.....	.....
10...	7.1	.....	.....	.....	0.0065	....	....	....	.....	.....	.....	.....
11...	7.1	.....	.....	.....	0.0075	....	....	....	.....	.....	.....	.....
12...	7.1	7.4	7.4	7.4	0.01	0.0129	0.0106	0.0082	0.4	1.2	2.5	1.37
13...	7.1	7.4	7.4	7.4	0.02	0.0215	0.0210	0.0210	1.0	1.7	4.3	3.33
14...	7.1	7.4	7.4	7.4	0.03	0.0364	0.0315	0.0252	3.0	1.7	6.7	3.80
15...	7.1	7.3	7.4	7.4	0.05	0.0451	0.0462	0.0420	3.7	12.0	16.0	10.57
16...	7.2	7.3	7.4	7.4	0.1+	0.0696	0.1075	0.1275	19.9	10.2	26.6	18.90
17...	7.2	7.2	7.4	7.3	0.5+	0.3440	0.4200	0.4200	70.1	76.5	82.2	76.37
18...	7.1	Lost	7.4	7.2	1.0	Lost	0.8500	0.8300	Lost	82.3	104.4	93.35
19...	6.5	6.6	6.8	6.7	5.0	0.0033	0.0033	0.0033	80.2	83.6	78.4	80.27

* There was no growth in solutions 1 to 11 inclusive. Included in this table are those solutions only for which determinations of initial iron content would be made with certainty. The initial pH of solutions 1 to 7 inclusive was 7.1 in each case.

and final iron determinations were made on the residual culture solutions. The data are presented in table II and fig. 1. It will be observed that the best growth was obtained with the higher iron concentrations, 5 and 1 mg. Fe per culture, although growth was nearly as good at 0.5 mg. Fe. Below this the growth was dropped sharply to zero at 0.007 mg. Fe per culture. The final pH determinations showed that all the solutions tended to become more alkaline, due probably to the utilization of NO_3 ions in excess of Ca or K. The final iron tests indicated that comparatively little iron was taken up by the alga during growth. In the lower concentrations of iron, where the alga crops were small, almost complete recovery of the original iron was recorded. In the cultures containing 5.0 mg. Fe

there was only the slightest trace of iron left in the solution, the bulk of it being precipitated.

Results of second experiment

The results of the first experiment, although consistent and apparently representative of the prevailing conditions, seemed to contradict our previous experience with the growth of *Chlorella* in several different nutrient solutions. In many former experiments only 0.02 mg. Fe per culture was actually added to the solutions, although this

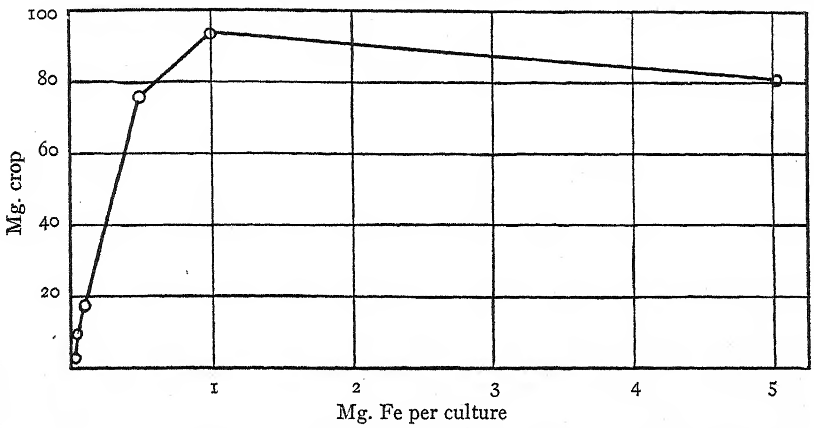

FIG. 1.—Effect of concentration of iron on growth of *Chlorella;* Experiment 1

amount was usually increased to about 0.05 mg. per culture by the iron present as an impurity, principally in the glucose. With this amount of iron, crops of 80–100 mg. were frequently produced in two weeks. In the experiment just described, however, such crops were attained only where at least ten times that amount of iron was present. In Experiment 6 of our earlier paper crops of over 100 mg. were produced on a solution with an initial iron content of 0.05 mg. per culture, and an initial pH of 7.5. In this case, however, calcium was omitted from the solution, whereas the solutions with differing iron content may have contained some calcium as a constituent of the basic iron-free nutrient solution. It is possible that an altered permeability of the cells to iron may have been produced by the calcium, thus explaining in part the apparently higher iron requirement in the latter solutions.

In the first experiment, just presented in detail, the cultures were maintained in the laboratory, where during the winter light may have been an important factor in the amount of growth obtained. It was decided, therefore, to repeat the experiment in the spring, growing the cultures in the greenhouse. The procedure adopted was exactly the same as in the earlier series, and all the reagents used in the preparation of the basic iron-free nutrient solution were from

TABLE III

GROWTH OF CHLORELLA IN SOLUTIONS OF DIFFERING IRON CONTENT; EXPERIMENT 2

SOLUTION NO.	FE ADDED MG. PER CULTURE	pH OF SOLUTIONS				FE IN CONTROL (MG.)	DRY WEIGHT OF CROP (MG.)			AVERAGE CROP (MG.)
		Initial control	Final A	Final B	Final C		A	B	C	
1........	0.000	7.3	7.3	7.3	7.3	0.000	......	......	......	......
2........	0.010	7.3	7.3	7.3	7.3	0.025	......	......	......	......
3........	0.040	7.3	7.3	7.3	7.3	0.055	......	......	......	......
4........	0.050	7.3	7.3	7.3	7.3	0.079	......	......	......	......
5........	0.075	7.3	7.3	7.3	7.4	0.092	0.2	0.0	1.0	0.26
6........	0.100	7.4	7.3	7.2	7.3	0.115	2.8	76.7*	12.5	5.10
7........	0.200	7.3	7.3	7.3	7.3	0.276	44.6	35.6	50.9	43.7
8........	0.300	7.3	7.2	7.3	Cont.	0.368	99.0	75.9	Cont.	87.4
9........	0.400	7.3	7.2	Cont.	7.2	0.552	74.9	Cont.	82.0	78.4
10........	0.500	7.3	7.1	7.2	7.1	0.736	Lost	86.5	90.8	88.6
11........	0.750	7.3	7.1	7.1	7.1	0.920	96.3	99.6	104.1	100.0
12........	1.000	7.3	7.1	6.9	6.9	1.150	108.9	105.6	115.7	110.1
13........	2.500	7.1	5.9	6.9	6.8	2.250	133.8	141.5	151.2	142.2
14........	5.000	6.5	Cont.	6.7	6.5	4.600	Cont.	107.0	120.4	113.7
15........	10.000	5.4	5.4	5.4	5.4	0.0125	002.5	001.6	003.8	002.6

* Omitted from average. The heavy crop in this case probably resulted from accidental iron contamination.

Cont. = culture contaminated.

the same reagent bottles. A new standard iron solution was prepared however.

STANDARD IRON SOLUTION.—This was made by dissolving 500 mg. of pure iron wire in dilute (1–9) redistilled HCl. This solution was saturated with chlorine washed through concentrated H_2SO_4. The solution was then evaporated to dryness on the water bath. The iron was taken up in 15 cc. redistilled HCl to which a little water was added, and the solution finally diluted to 1000 cc. in a pyrex volumetric flask. One cc. of the standard solution contained 0.5 mg. Fe.

SODIUM CITRATE SOLUTION.—In this experiment a higher concentration of iron (10 mg. Fe per culture) was introduced into the series. It was therefore necessary to increase the dose of sodium citrate in order to hold this amount of iron in solution. For this purpose 3 cc. of a 5 per cent solution of the recrystallized salt was added to each culture flask.

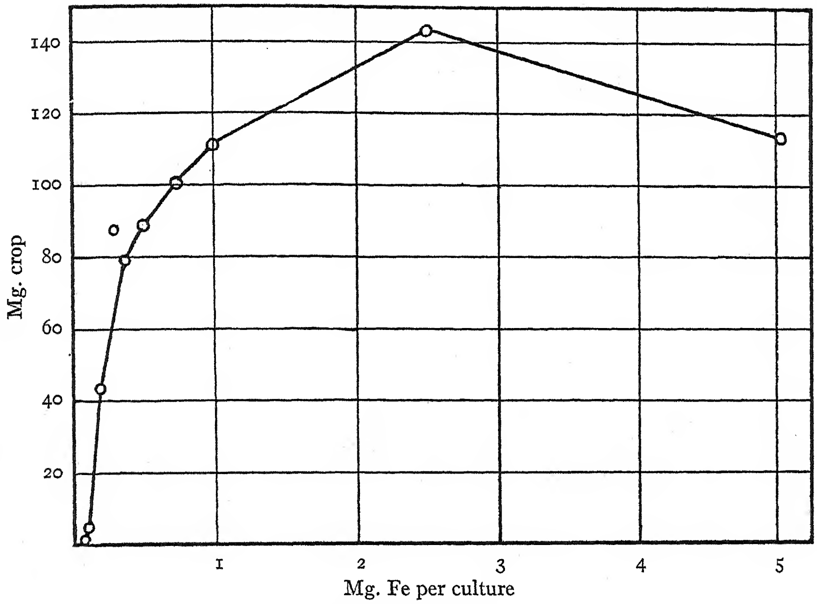

FIG. 2.—Effect of concentration of iron on growth of *Chlorella;* Experiment 2

PREPARATION OF SOLUTIONS OF DIFFERING IRON CONTENT.—Pyrex Erlenmeyer flasks of 150 cc. capacity served as culture containers. Following the addition of the sodium citrate solution to each flask, iron was added to each of four flasks for each of the concentrations given in table III. The volume was brought up to 25 cc. in each flask by the addition of the necessary amount of distilled water. The flasks were then sterilized.

After the introduction of 25 cc. of sterile basic iron-free nutrient solution to each of the culture flasks, three of each iron concentration were inoculated with a suspension of *Chlorella* cells, the fourth in each case being reserved as a control for pH and iron determina-

tions. The cultures were inoculated on June 21, 1926, and immediately placed in the greenhouse under partial shade.

At the end of two weeks no growth was observed in any of the first four groups of cultures, with iron content ranging from 0 to 0.05 mg. Fe per culture. In the remaining cultures the amount of growth apparently increased markedly with an increase in the iron content, from a "trace" in the solution with 0.075 mg. Fe to a very good growth in solutions with 1, 2.5, and 5 mg. Fe per culture. In the highest concentration of iron (10 mg.) no growth could be detected, but a heavy flocculent precipitate was present. When this precipitate was dissolved with a little HCl, it was found that the organism had made a slight growth in this solution.

Crop determinations were made in the usual way on July 6. Determinations of the final pH and iron content of the culture solutions were made at this time, as well as analyses of the "control" solutions. The data are presented in table III, and the relation of amount of growth to iron concentration is shown graphically in fig. 2.

Discussion

In the preceding experiments advantage was taken of the fact brought out in our previous work that iron can be almost completely removed from solution in certain culture media by adsorption on the calcium phosphate precipitate formed in alkaline solutions. It is probable that the high concentration of the phosphate ion in our solutions also aided in decreasing the solubility product for both $Ca_3(PO_4)_2$ and $FePO_4$. The method is suggested as a convenient means of freeing culture solutions from iron. Once having this iron-free solution, one may then proceed to study the effect of known amounts of iron added to the cultures on the growth of the organism under consideration.

Previous methods have usually depended upon the careful purification of the water, individual salts and organic components which enter into the composition of the culture medium by recrystallizations, redistillations, etc. (see MOLISCH 12 and CURRIE 3 for examples of this procedure). Objections to this method might be brought up; first, slight amounts of iron in the component parts of the solution which remain even after purification will be added

together when the solution is prepared; second, manipulation of the purified reagents would allow chance for contamination with iron.

Using the adsorption procedure, it was found that the iron was removed to such an extent that it could not be detected by our method of determination, and also so that there was insufficient iron for growth of *Chlorella*. Although, as will be shown later, the minimum iron concentration for this organism has not been found, it is thought to be low. Several difficulties present themselves in the use of such an iron-free solution for the investigation of the effect of iron concentration. The adsorption is of necessity carried out at an alkaline reaction, and if an iron salt is then added in varying amounts to portions of the iron-free solution it is rapidly precipitated as $FePO_4$. If one wishes to overcome this difficulty by shifting the reaction to a more acid one, he may possibly add iron in the acid used. And granted that this is done with an iron-free acid, iron will precipitate slowly at most acid reactions which are at pH values above the acid limit for growth. The iron would therefore be variable in each of the cultures during the course of the experiment, and the results would have no meaning.

In our work we attempted to obviate this difficulty by adding sodium citrate to the culture solutions. This we knew from our previous experiments (7) would keep the iron in solution, even at alkaline reactions. When the sodium citrate was added to the culture solution before adsorption of the iron impurities, however, it was found that it was also removed to a large extent by adsorption. When added afterward there was, of course, the possibility of adding some iron impurity which might affect the results at the low iron concentrations. The principal objection to the use of this salt may be its effects on the dissociation of the iron salt. This will be discussed later.

The change in color in iron solutions when an excess of sodium citrate is added to them seems to show that the iron changes into another form. It is usually stated that a complex organic salt of iron forms. What occurs might be considered to take place as follows: the addition of the sodium citrate to a ferric chloride solution of a pale straw color changes it to yellow, probably the color of the molecular form of ferric citrate. An excess of the citrate deepens

the color by decreasing the dissociation and giving more of the molecular form. This would be analogous to the effect of an excess of KSCN on the ferric sulphocyanate reaction. The iron apparently is true solution and not in a colloidal state, for it is not precipitated in the presence of the sulphate and phosphate ions which are in the culture solutions. That the sodium citrate does not act as a protective agent is evident from tests made by the writers that showed colloidal iron solutions to be precipitated by sulphate and phosphate ions, even in the presence of sodium citrate.

Both the high amount of iron present at the lower limit for growth and the large amount necessary for good growth in these experiments are hardly in keeping with results of other workers or with our previous experiments. Taking the results as they stand, however, in Experiment 1 we have a rapid increase in growth as the amount of iron increases from the limiting concentration of 0.0075 to 1 mg. per culture. An optimum concentration is not well defined, but at 5 mg. per culture there is a falling off in the growth. In Experiment 2 the results are similar; the curve rises rapidly from a limiting concentration, in this case of 0.075 mg., to an optimum concentration at about 2.5 mg. per culture, and drops again at 5. At 10 mg. there is practically no growth, but this is due to loss of iron by precipitation, as the table shows, and is probably not due to toxicity. There was too little sodium citrate to keep this large amount of iron in solution. Whether the smaller crops in both experiments at 5 mg. of iron per culture is the result of toxicity is uncertain, but it should be pointed out that in both cases there was a distinct change in the reaction of the culture medium caused by the amount of acid added with the standard iron solution, in spite of the fact that the culture solutions were strongly buffered. The conditions involved are rather complicated, and it will require further study to elucidate matters.

One thing difficult to explain has been the difference between the amount of iron needed for good growth of *Chlorella* in the present work and in our previous experiments (7) on the effect of pH on growth. In the latter experiments, as has already been mentioned, good crops of the alga were obtained at alkaline reactions when only 0.05 mg. of iron per culture (50 cc.) was used; for example at pH

7.15, 83.4 mg. were obtained (see Experiment 6 of that paper), while in the present experiments very little growth was obtained at this concentration, namely, 10.6 mg. in Experiment 1 (pH 7.1–7.4), and none in Experiment 2 (pH 7.3). It was at first thought that the difference might be explained by the fact that a calcium salt was used in the preparation of the culture solutions in the second case and not in the first, and that the presence of calcium might either decrease the ionization of the iron salt in some manner, or more likely change the permeability of the cells to iron. This is probably not true unless the calcium acts in very minute amounts to produce this effect, because a rather sensitive qualitative test failed to show the presence of calcium. It had apparently precipitated out quite completely as the phosphate in the preparation of the iron-free solution.

Since performing these experiments and writing most of this paper, some investigations by USPENSKI which have been called to our attention seem to give a better explanation of the differences found. USPENSKI (15) considers that when certain organic substances, such as sodium citrate (or tartrates as in Raulin's medium), are present in culture media the iron enters into complex organic combination, and in such state is only slightly ionized and hence only available in low concentration. It might appear therefore that large quantities of iron were necessary for growth under these conditions, while actually only a small amount of what is present is active. The solution acts as a buffer as regards iron. USPENSKI also shows that the addition of citrate markedly decreases the intensity of the reaction with KSCN. If, for example, at pH 3.4 the reaction of a given iron solution without citrate is taken as 100, the addition of 0.000005 M citrate decreases the intensity to 80 and of 0.00005 M citrate to 25. In another paper USPENSKI and USPENSKAJA (16) state that in the presence of 0.004 M sodium citrate the most favorable concentration of iron for *Volvox* is from 2.5–5 mg. Fe_2O_3 per liter, while in the pure nutrient solution without citrate 0.5–1 mg. Fe_2O_3 per liter is best. The pH was 7.6. The important and interesting idea they put forth is that it is not the total amount of iron present but the amount of iron-ion which is active.

In our experiments on iron greater amounts of sodium citrate

were used than in those concerned with the effect of the H-ion, and in the second experiment more citrate was added than in the first, and it is possible in these cases that because of the high citrate content we had decreased the Fe-ion concentration to a low value, although the total amount of iron was large. In Experiment 6 of

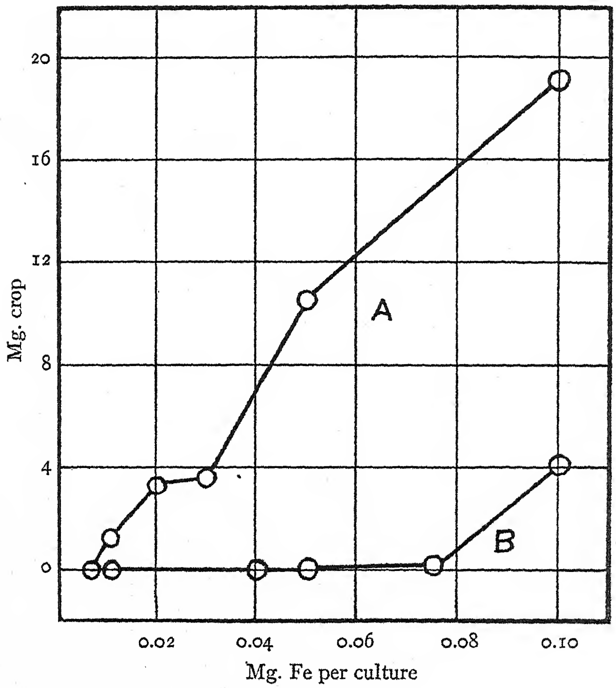

FIG. 3.—Limiting concentrations of iron for *Chlorella* plotted to larger scale: *A*, Experiment 1 in which low concentration of sodium citrate (0.04 gm. per culture) was used; *B*, Experiment 2 in which high concentration (0.15 gm. per culture) of sodium citrate was used.

our first paper 0.01 gm. of sodium citrate per culture was used. In Experiments 1 and 2 of this paper 0.04 and 0.15 gm. respectively were used. This appears therefore to explain the large differences found. Fig. 3, in which the data for Experiments 1 and 2 for small amounts of iron are graphed on a larger scale, shows strikingly this difference. The limit for growth with the greater amount of citrate

is at 0.075 mg. of iron per culture, with the smaller amount at 0.0075, or about one-tenth as much.

These results lend support to the ideas of USPENSKI that it is not the total amount of iron in the culture medium, but the amount in the ionized form which is effective physiologically. The iron is of course assumed to be in solution. Further, our results do not show a limiting and an optimum iron concentration for growth of *Chlorella*, but only the limiting and optimum concentrations for a given set of conditions. Indeed, will it be possible to determine this except for particular iron salts in given culture media? The effect of the iron-ion would give perhaps more consistent results. The case is analogous to the effect of total acidity and of H-ion concentration on plant growth. It is probable, however, from the results of these experiments, although no knowledge is at hand in regard to the extent of ionization in the solutions used, that the iron-ion concentration for good growth of *Chlorella* is small. It is interesting to note that although this may be true, we are able to remove the iron by adsorption below the limit where growth will occur. This is shown in these experiments by the absence of growth in the "iron-free" nutrient medium to which was added neither sodium citrate nor iron.

Summary

1. There are no fundamental data on the iron requirements of plants. This is true of both minimum and optimum amounts. This lack of information is due in some cases to failure to consider the solubility of the iron added to culture solutions, and in others to failure to remove completely all the iron present as impurities in such solutions.

2. We have presented a method of removing from culture solutions apparently the last traces of iron by means of adsorption at alkaline reactions, and have further shown that if sufficient sodium citrate is used in solutions free from precipitate, iron added subsequently will remain in solution indefinitely.

3. In an attempt to use these methods to determine the relation of iron to the growth of *Chlorella*, a high minimum concentration was found which varied in the different series, and was higher in those which had a higher sodium citrate content.

4. The hypothesis put forth in explanation of these results is that iron is active in growth only in the ionized form, and that increasing the amount of citrate decreases the ionization of the ferric citrate present in these solutions. Therefore, while we may have a larger total amount of iron present, we may have little or no growth because of a low concentration of the ferric ion. This explanation is in keeping with the work of USPENSKI.

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ECOLOGY, PLANT GEOGRAPHY, AND GEOBOTANY; THEIR HISTORY AND AIM

EDUARD RÜBEL

Introduction

Are ecology, plant geography, and geobotany three different sciences, or three different viewpoints of the same science? Is one a part of the others, or are all three synonymous? Opinions on these questions differ widely, and the writer thinks it worth while to review the history and discuss the matter. The necessity of discussing it again was emphasized at the Ithaca Congress. It was evident there that ecology is often understood in a rather narrow sense by those not especially interested in it; while American ecologists use it in a very broad sense, applying the term to all their work and intending it to embrace also chorological, chronological, and genetical phases of botany and zoology. Reducing the term to a narrow meaning when it is meant in a broad one brings it into a somewhat wrongly isolated position, especially unwelcome in our times of over-specialization. To correct misunderstandings by non-ecologists, I wish to discuss what ecology includes in the minds of ecologists, what plant geography means to plant geographers, and what geobotany comprehends, giving also the other narrower sense of each term.

Ecology,[1] from *oikeo* to dwell, can include all science about dwelling, environment, household. The whole earth, with its climates, soils, and bions, is environment to the plant or the plant community; so that the study of all factors, static and dynamic, and all the regional influences on all beings distributed over the earth is ecology: plant ecology, and animal ecology including human ecology or ethnography.[2]

Geography is the study of the earth, of its inorganic part in

[1] The term originated from HÄCKEL in his *Generelle Morphologie der Organismen*, 1866, as the science of relationships of organisms to environment (Wissenschaft von den Beziehungen der Organismen zur Aussenwelt).

[2] The section of Ecology in the Congress of Plant Sciences was meant in this wide sense, but four such ecological papers were read at the same time in the section of Taxonomy.

physical and morphological geography, of its organic part in plant, animal, and human life, especially economic geography. One must consider how and why the beings can and do live on the earth, how they must be distributed, and how they react; if the beings under consideration are plants or plant communities, the study is plant geography.

Geobotany is that part of botany which has to do with *gea*, the earth, the action of all earthly factors, all changes of plants on the earth, all distributional questions over the earth. GRISEBACH (6) wrote as long ago as 1866 "Die auf die Geographie der Pflanzen gerichteten oder, wie man sich kürzer ausdrücken kann [not only shorter but I should say more precisely and accurately], die geobotanischen Forschungen haben die Aufgabe," etc. Later on he discusses "Allgemeine Geobotanik" and "Spezielle Geobotanik, die Vegetation der einzelnen Länder." Everyone will admit that taken in this broad sense the three terms are synonymous.

Yet other synonyms of our science are "history of plants"[3] WILLDENOW 1792 (23), and "phytostatic" THURMANN 1849 (20). WILLDENOW gives the following definition:

Unter Geschichte der Pflanzen verstehen wir den Einfluss des Klimas auf die Vegetation, die Veränderungen, welche die Gewächse wahrscheinlich erlitten haben, wie die Natur für die Erhaltung derselben sorgt, die Wanderungen der Gewächse und endlich ihre Verbreitung über den Erdball.

And THURMANN writes:

La géographie botanique présente le tableau des faits de dispersion végétale et les met en rapport avec leurs causes. L'expression de phytostatique qui a déjà été employée par quelques observateurs satisfait bien à cette définition. Elle est plus générale et plus exacte que celle de géographie botanique qui, en éveillant particulièrement l'idée descriptive dans de grandes proportions, n'implique aussi bien, ni le point de vue topographique, ni le côté spéculatif de l'étude stationelle.[4]

Le sens essentiellement stationel que l'on a attaché au mot phytostatique est donc en réalité tout-à-fait légitime et conforme à l'étymologie.

So these two terms mean the same thing as the three we are principally considering. What do they embrace within botany?

[3] "History" is nowadays still often used in the sense of description (story from *storia*, a late Latin derivation of the ancient Latin *historia*). Also "natural history" comprises the whole science of nature, not only a historical portion of it.

[4] Phytostatique includes station, habitat (climate and soil).

Historical and logical branches of biology

The beginning of pure botany was a general description and classification of the exterior of plants. Thus these are the beginnings of morphology and taxonomy. Later on arose the questions about life-processes, physiology. When the microscope was invented, morphology was enlarged by anatomy, the study of the micro-forms seen after making cut sections (*anatemno* to cut). For a long time these three phases of botany prevailed. Much later geographical, ecological, palaeontological, and genetical questions arose, and grew to sections of plant sciences of equal standing.

Instead of this purely historical sequence of branches of botany, TSCHULOK (21) gave a logical one considering what is necessary and sufficient ("notwendig und hinreichend") to cover all physical knowledge about living beings. There are seven incommensurate material points of view.

1. Taxonomy, the science of relationship: the distribution of the plants in groups according to their degree of similarity. These are species, genera, families, etc.

2. Morphology, the science of form: the study of the outer and inner (anatomical) form.

3. Physiology, the science of the processes of life.

4. Ecology, the science of the household: the adaptation of organisms to the external world, the earth; the arrangement of the household.

5. Chorology, the science of space: the distribution of the organisms in space (on the earth).

6. Chronology, the science of time: the chronological appearance of the organisms in earth history.

7. Genetics, the science of development: the descent of organic beings.

Our science is not one of these. It has a compound point of view, and may be defined as follows:

Geobotany (plant ecology—plant geography) is the science of the relationship of plants to the environment, the earth.

Several of the seven points fall into this definition. Morphology and physiology concern form and function of the single plant and its organs, and thus can be considered without necessarily knowing the

environment. Taxonomy groups the *Sippen* without necessary relation to surrounding land, but chorology asks how plants are distributed on the earth and how they migrate; ecology endeavors to determine how plants arrange their household on the earth within the given climate, soil, and conditions of competition. Chronology considers the bearing of the plant to time, to earth periods. This chronological or historical viewpoint is intimately connected with genetical evolution. The earth periods in which the plants grow are subject to change; at the same time the plants themselves change under these acting forces. The geogenetical question of chronology is always united with the phylogenetical one of genetics proper, and forms evolutionary botany.

Three groups of problems

Since environment acts in different directions and postulates different questions, we must distinguish in geobotany three great groups of problems:

1. The problem of space, the question of the distribution of plants on the earth.

2. The problem of habitat, the organization of the household of plants, the question of behavior toward habitat in the widest sense.

3. The problem of change, the question of plant behavior toward the changes of the earth and the changes of the plants themselves in time.

Of course the different problems must not always be considered separately; on the contrary in any paper all have to be kept in mind, even when one of them predominates in the study. Often they combine necessarily. In the historical consideration of Tertiary or Pleistocene plants there is also a problem of space and habitat. Although we treat the Pleistocene and Tertiary plants in genetical (historical) geobotany, because they lie far behind in the genesis of the earth, there nevertheless exists a chorological geobotany of the Pleistocene, an ecological geobotany of the Pleistocene.[5]

[5] In the lecture programs of the University of Zürich one finds that in 1911 and several times in later years courses were announced: BROCKMANN-JEROSCH: Pflanzengeographie des Diluviums (an extension of the field in accordance with COOPER'S wishes of 1926 ([2, p. 393]).

Two objects of research

Thus far we have always spoken of "the plant" as the object of research (not meaning the individual, but the abstract *Sippe*), in the first instance the species, but also the genus, family, etc., all of which are entities recognized in the *Sippen* of taxonomy, forming the flora. The other object of botanical research is a sociological one, the study of the plant community, which is composed of quasi-organisms that follow their own laws. Under given circumstances of climate, soil, or competition certain groups of plants are always found living together, forming a well defined object of research. This research is plant sociology or the study of vegetation. The term plant sociology is so satisfactory, so to the purpose, that it originated independently in the minds of workers in Germany, Russia, Sweden, Switzerland, and the United States (1, 13).

In the analysis of the plant community we need to know the habitat, the physiognomy (life-form), and the composition, the flora which characterizes a certain area. Historical causes (genetic factors, immigration) account for the presence of the species in this given area. The habitat of itself is not botanical; the study of habitat becomes botanical when its effect on vegetation is considered. The vegetation is seen through its physiognomy, especially the part resulting from adaptation. External description of landscape is not scientific botany; the student of physiognomy must penetrate into the details, which include the constitutional characters of species and also epharmosis, adaptation to the environmental conditions which form the habitat.

The habitational point of view began with LINNAEUS, and was taken up by SCHOUW, HEER, and FLAHAULT; the physiognomical one dates from WILLDENOW, HUMBOLDT, and GRISEBACH. The combination of the two lines which alone can bring about real sociology began in the fifties and sixties of the past century (13),[6] in the works of SENDTNER, LORENZ, KERNER, and GRISEBACH. We find it spendidly carried through in 1895, in WARMING'S (22) *Plantesamfund*, literally translated "plant communities."

[6] So I would speak of seventy years of phytosociology and not of twenty-five (ALECHIN 1). The science of phytosociology is as old as that, only the word is younger.

The combination of the three large geobotanical problems and the two objects of botany may be tabulated as follows.

THE SIX RESEARCH BRANCHES OF GEOBOTANY (OR PLANT ECOLOGY OR PLANT GEOGRAPHY)

		PLANT *Sippe;* FLORA	PLANT COMMUNITY; VEGETATION
Problem of space	Plant geography	Autochorologic geobotany	Chorologic sociology
Problem of habitat	Plant ecology	Autecologic geobotany	Ecologic sociology
Problem of change	Plant history	Autogenetic geobotany	Genetic sociology (study of succession)

Aims of the six branches

Each branch may be studied in different directions by starting from the problem or from the object. Each branch may be connected with any other one and combined for new viewpoints, new knowledge.

A. PLANT GEOGRAPHY OR CHOROLOGY[7]

1. CHOROLOGIC OR GEOGRAPHIC STUDY OF THE FLORA, AUTOCHOROLOGIC GEOBOTANY.—Floristics belong to this branch. Starting from the object, the plant *Sippe*, one studies the distribution of each species, genus, and family, determines its area, and how it migrated to occupy it. Starting from the problem of space one surveys the flora of a given district. From these floras we learn to divide the globe into floral regions, floral provinces, districts, etc.

2. CHOROLOGIC OR GEOGRAPHIC STUDY OF VEGETATION, CHOROLOGIC SOCIOLOGY.—Starting from the object, the distribution of every plant community recognized over the earth must be studied. More often a local area or district is selected, and in the form of a monograph all communities of this district are determined and surveyed. In applied form we consider where different crops may be grown, the distribution of plant communities indicating the possible distribution of crops.

[7] Logically chorology is a better term for the study of distribution, as geography comprises the study of everything (plant distribution, environment, changes) concerning the earth.

B. Plant ecology

3. Ecologic study of flora, autecologic geobotany.—The habitat of the plant is studied, the means of distribution of the plant and its adaptations; its need of light, moisture, temperature, of soil contents, of competition, the H-ion concentration, etc. The result is the success of a plant in this or that habitat, its growth in a certain adapted life form (supplemented by its constitutional forms).

4. Ecologic study of vegetation, synecologic geobotany, ecologic sociology.—What was considered in the ecology of the single plant is considered here for the whole community; that is, its habitat, including climate, soil, competition, action of animals and man. Large parts of political economy belong in this branch. Forestry is the applied ecology of the plant communities called forests, and in agronomy applied ecology teaches the habitat for corn and fodder growing, etc.

C. Plant history

5. Genetic study of flora, autogenetic geobotany.—In the problem of change of the plant we study the phylogeny of species, and combined with the problem of space how the new *Sippen* created by evolution spread into their area. Starting from the changes of the earth the endeavor is made to follow the development of the flora through the geological periods, some species dying out and others newly coming into existence. The history of every flora and the evolution of species are two great questions of this branch.

6. Genetic study of vegetation, succession of vegetation, syngenetic geobotany.—We study how the plant community comes into existence and how it changes into another one. If the factors which bring about the change are of biotic nature we speak of biotic successions (Cowles); if it is erosion or alluvium we call them topographic successions; when they are conditioned by change of climate of different geological periods they are regional (climatic) successions. Starting from the changing periods of the earth and combining with space and habitat, we study the plant communities of the Tertiary, the Pleistocene, etc.

Combining our branches, nos. 1, 3, and 5 form the study of the flora, all geobotanical problems of the single plant or *Sippe*, the

autogeobotany. Nos. 2, 4, and 6 form the syngeobotany or phytosociology.

To know the plant communities we need to study, besides the main geobotanical problems, the community itself: its morphology determined by its floristic composition, abundance, constancy, and fidelity (and various other minor features) of each constituent, etc. (RÜBEL 14, 15). Of course a taxonomy of communities is necessary also, and in the ultimate result a system, because we cannot be satisfied by juxtaposition of many results without erecting a philosophical structure of the whole.

History of terms

Having displayed the logical branches of our science and their aims, a few terms must be followed up historically to show how narrower and wider senses arose which led to misunderstandings.

As mentioned, "plant geography" was for a long time and still is the term widely used for comprising our whole science in question, so far as known. With its development it had to be subdivided. Three sections have long been recognized. WILLDENOW (1792) in his history of plants recognized one entity, HUMBOLDT (1793) in a footnote for the first time says that *Geographia plantarum* must be carefully distinguished from *Historia plantarum*. SCHOUW (1822) definitely speaks of plant geography as dealing with present conditions of plants on the surface of the earth, plant history as considering the origin and changes of plants, and plant physiology as the field from which must be learned the action of the external factors on plants. Later on the three fields were generally named floristic (chorologic) plant geography, ecologic (formerly physiologic) plant geography, and historic (evolutional) or epiontologic plant geography.

Basing on physiognomy (growth forms—life forms), and especially on ecology, the recognition and study of plant communities (vegetation, meant in opposition to flora) grew more and more. Plant communities cannot be separated from their cause, the habitat. The ecological idea lies in the concept of plant community. So when WARMING wrote his *Plantesamfund* (plant communities) he gave it the main title of *Lehrbuch der okologischen Pflanzengeographie* or

Grundträk af den ökologiske Plantegeografi. Therein he treated all the new science of plant communities, and also the ecology of the single plants, the species. This has long persisted of course, and is still often spoken of as ecological plant geography, including of our six branches nos. 2, 3, 4 and 6. In WARMING's English edition *ökologische Pflanzengeographie* was translated by the short word ecology. So ecology there meant the four branches just cited, but it even extended to the still wider meaning of all six branches.

How did "ecology" come into its position? In plant geography in the first instance geographic, floristic questions were treated. Later on, when ecological points of view deepened the study considerably, not all workers were quick in taking up these new viewpoints. In order to emphasize the deepening by ecological studies, the British vegetation committee laid stress on the term ecology in opposition to mere descriptive floristics. It soon was accepted in the English language for a whole group of geobotanical research branches; many took it for all six (the study of flora and of vegetation); some, according to WARMING's translation, for the four branches mentioned above; others even for the three branches of the study of vegetation.[8] I prefer to use it only for the two branches of autecological geobotany and synecological geobotany or ecological plant sociology. TAYLOR (19) is right when he objects to using ecology without botanical prefix when only botanical questions are dealt with and not zoological ones as well. We never omit "plant" before geography, but often omit "plant" before ecology. Historically this is easily understood. Geography was first used for physical and political geography, and these are cited as geography without prefix. Sometime later the botanical branch of geography was added, the new branch characterized by the prefix "plant." In ecology it was the reverse. The word came into general use through the English and American botanists and WARMING's translation, so this was used without prefix. Later on animal ecology began to become

[8] COOPER (2) p. 395: "Ecology is the science which treats of the vegetation of the earth." Citing this page of COOPER's most interesting paper, I may refer to an omission. COOPER says about works on succession on the European continent: "Two papers have appeared which deal exclusively with succession, namely, by LÜDI (1919) and FURRER (1922)." Six years earlier (1913) SIEGRIST (18) published his paper dealing exclusively with the successions of the alluvial forests near the Aare.

prominent also. We must make allowance for this fact and use the term ecology without a prefix only when animals are included as well as plants. I agree with TAYLOR[9] that phytosociologic concepts are just as applicable to animals as to plants. GAMS (4) has taken the same view as urged by TAYLOR and JONES (8), and has included animals as well as plants in his system of communities (*Biocoenosen*), especially in his *Versuch einer Übersicht über die Lebensformen des gesamten Pflanzen- und Tierreichs* (*l.c.* p. 339).

After the International Botanical Congress of Paris in 1900 had asked authors to make propositions for phytogeographical terminology, SCHRÖTER (16) proposed for the study of plant communities the term *Formationslehre* or synecology, as the study of the plants which dwell together. The field of synecology he divided into physiognomical synecology, geographical synecology, ecological synecology, and historical (*florengeschichtliche*) synecology. Ecological synecology shows the word ecology in the adjectival form for the research problem; in the substantive form for the object of research. Accordingly, autecology, the ecology of the single plant, does not correspond with synecology, the whole study of communities, but only with ecologic synecology, the ecology of plant communities. For the time being then, SCHRÖTER's term synecology was certainly excellent and served the purpose of advancing the science. But since then this study has deepened considerably and spread widely within all its problems of space, habitat, and change, so as to make it more advantageous to keep synecology for the habitat problem of vegetation, and call the whole science of plant communities, as said before, plant sociology.

Summary

Logically we have in geobotany three great problems of research: space, habitat, and change; and two objects of study: the plant and the plant community. That gives us six branches of our science: (1) autochorologic geobotany; (2) autecologic geobotany; (3) autogenetic geobotany (combining with the study of the flora); and (4) synchorologic geobotany or chorologic sociology; (5) synecologic geo-

[9] I am glad also that TAYLOR emphasizes the great importance of biotic factors; BROCKMANN-JEROSCH and I have always done this as much as possible in most of our papers.

botany or ecologic sociology; (6) syngenetic geobotany or genetic sociology, study of succession (combining with the study of vegetation or plant sociology).

Historically plant geography, plant ecology, and geobotany are synonymous and include all six branches. Geobotany (GRISEBACH) always does this; the two other terms are ambiguous, because often used in narrower and wider senses. The different meanings are discussed.

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CONTROL OF SEX REVERSAL IN THE TASSEL OF INDIAN CORN[1]

JOHN H. SCHAFFNER

(WITH THREE FIGURES)

Zea mays is an example of the more extreme type of monoeciousness exhibited by various species of angiosperms, since normally the whole inflorescence and a considerable part of the stem and leaf structures below both the ear and the tassel show an evident secondary, sexual dimorphism. It is, therefore, a very favorable species for the study of the influence of sexual states in determining sex-limited morphological characters. Because its monoecious condition is apparently of recent origin, phylogenetically considered, it is also a favorable plant for the study of the control of the time of sex determination in relation to the ontogeny. Theoretically it would appear that the dioecious plants should be the type in which sex reversal could be brought about most readily, because in these plants there is the longest possible period in which to influence or control the various ontogenetic gradients present in the developing system.

Having found, by various experiments and observations on dioecious plants (SCHAFFNER 6, 7, 8, 9, 10), that the sexual states were determined by functional gradients rather than by differential heredities, and that in some of them sex reversal could be easily induced by simply changing their functional states through the influence of some external environment, and having also studied a number of monoecious species (7, 10) to determine the relation of their functional gradients to sex determination, the writer decided to find how much change could be produced directly in Indian corn through an attempted control of its sexual states, its points of sex determination, and its possible sex reversals. In a monoecious plant like corn, the disturbance of the metabolism, with probable consequent changes in the carbohydrate-nitrogen ratio (GARDNER 2), and other such conditions in the cell complex resulting in changed sexual

[1] Papers from the Department of Botany, The Ohio State University, no. 205.

states at the given points of the determinate growth gradients, should be nearly as easily produced as in dioecious species. This is obvious since the cell development and cell lineage, from the beginning of sex determination in the stem to the production of spikelets or flowers, involves a considerable part of the ontogenetic cycle. To cause a disturbance or a change in the sexual conditions of a plant with bisporangiate flowers of the more extreme type, with its greatly shortened floral axis, in which the determinate gradient is completed, accompanied by the passage from a neutral state to a male or to a female state, would presumably be enormously more difficult because of the great difficulty involved in getting any control of the functional processes in the minute length of axis which is developed before the gradient ends in complete determination. GARDNER (2) was able to induce changes in the sex condition of the Senator Dunlap strawberry, which has bisporangiate flowers. The strawberry belongs to a dioecious group, however, and the Senator Dunlap must be considered as having evolved to the point of dioeciousness or returned to the bisporangiate condition through a recent mutation.

Many monoecious plants which would otherwise be favorable as experimental material are difficult to manipulate because of their geophilous habit. One difficulty with Indian corn is that the inflorescences are completely covered with sheaths or husks until the flowers reach maturity. *Sagittaria*, which might otherwise be very suitable for experimentation, is hydrophytic and thus requires special greenhouse facilities. Corn is on the whole easily cultivated, and because it is greatly influenced by the ordinary environmental factors, it was thought that it ought to be a favorable object for simple experimentation, and that there should be no difficulty in influencing its functional gradients which have their play during the normal ontogeny of the plant. Since sex reversal occasionally occurs in the tassel under ordinary field conditions, apparently the easiest changes would be the reversal of the tassel to partial or complete femaleness, and the shifting of the point of female determination up and down the main stem, which normally takes place only in the incept of a lateral branch. This was therefore selected as the first point of attack, since it was already known that the short light

period had a decided influence in reducing the size of the plant and its photosynthetic surface.

Preliminary tests were made in the winter of 1925–26. On December 14, 1925, sweet corn of the variety Narrow-grain Evergreen was planted on shallow greenhouse benches in soil abundantly supplied with manure and water. There were 28 plants in the plot, spaced about 6 inches apart. Since corn is a slow-growing plant which is not hastened to maturity by a short photoperiodicity to any appreciable extent, the days were already lengthening considerably when flower production began. Nevertheless 21 of the 28 plants in the plot produced tassels with a greater or less degree of carpellateness, resulting in silk and grain development. Thus under the special conditions, this plot of 28 plants showed 75 per cent of the individuals with sex reversal to femaleness in the tassel, and 25 per cent normal monoecious individuals with the tassels in the pure male state.

Another plot was planted December 17, 1925, with Country Gentleman sweet corn which had been selfed for two years by MARION T. MEYERS of the Farm Crops Department, to whom the writer is indebted for the seed. This plot contained 55 plants. Of these 44, or 80 per cent, showed sex reversal in the tassel, and 11, or 20 per cent, showed pure staminate tassels. The results were thus practically the same for the two varieties. It was evident, therefore, that a decided control of sex expression could be accomplished with the development of a suitable program.

During the summer of 1926, Narrow-grain Evergreen sweet corn was planted in the garden with the ordinary spacing for plants in the field. A large patch was studied for evidence of sex reversal in the tassel, but all the tassels, amounting to several hundred, were found to be pure staminate. Under normal field conditions reversal in the tassel must be quite rare in this variety. The development of silks and grain in the greenhouse plants, therefore, was due to the short light period in combination with the other environmental factors. It was noted that those plants which had sprouted somewhat later than their neighbors, and were thus soon somewhat shaded, not only remained quite small in comparison with the others but also showed the greatest degree of sex reversal.

With this preliminary knowledge of the apparently efficacious environmental factors, an experiment was planned for an attempted complete percentage control of sex reversal in the tassel. A tank 3 feet deep and 3 feet wide was available, and in this Narrow-grain Evergreen sweet corn was planted in well manured soil, November 1, 1926. All of the 20 plants of this plot showed sex reversal in the tassel. There was therefore 100 per cent control, so far as the possibility of reversal was involved. The degree of reversal was of varying

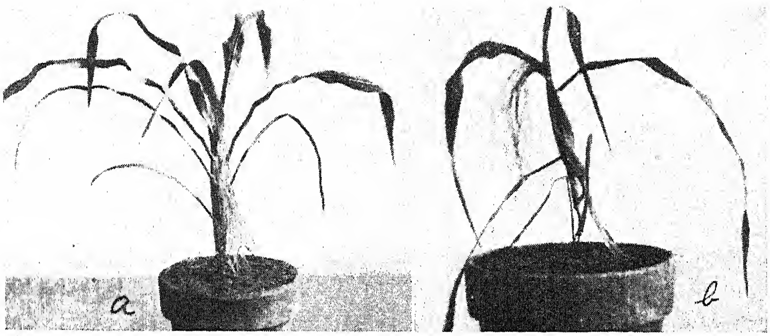

FIG. 1.—*a*, completely reversed plant, showing abundant silks developed from terminal inflorescence and reaching ground, taken before crimping of stem; *b*, extreme type of carpellate plant, showing flexuous condition beginning to appear at second node and forcing leaf sheaths apart; both plants transferred to pots for photographing.

intensity and completeness, ranging all the way from tassels with a few silks and developing grains among the staminate flowers, up to those which were completely reversed and showing a fairly well developed cob with no staminate flowers whatever. The 20 plants began blooming January 25, and the blooming period was completed February 15, when the last plant began to protrude silks from the terminal sheaths. The most extremely carpellate plant was exactly 6 in. high (15 cm.) to the point at which the silks emerged from the leaf sheaths, and had just 6 nodes and leaves (fig. 1 *b*). The taller, less extremely reversed plants had as many as 12 leaves.

On February 14 all the plants except one had been removed from the plot; so it was immediately replanted with Narrow-grain Evergreen sweet corn in order that a second crop might be obtained, developed under the influence of the lengthening days of spring.

There were 25 plants in this second plot, and of these only 3 showed sex reversal to a slight degree, or 12 per cent. Of the 3 plants with reversal in the tassel one had 3 silks and the other two had 2 silks each. The leaves of the plants of this plot ranged from 10 to 15. Evidently the longer light had a decided influence in promoting maleness in the inflorescence.

During the winter several plots of the Narrow-grain Evergreen variety were planted also on shallow benches. These behaved much like the plants of the previous winter, and were less extreme in sex reversal than the plants in the deep soil in the tank. One plot of 17 plants, planted November 2, 1926, showed 13 with reversed tassels, or 76.5 per cent. Another planted November 16 had 11 plants, 8 of which showed reversal, or 72.5 per cent. A third plot, part of which was destroyed by mice, was planted December 17, and of the nine surviving plants five, or 55.5 per cent, showed sex reversal in the tassel. The shallow soil of the benches was apparently less favorable for producing sex reversal than the deep soil in the tank.

Some of the plants from the benches were studied for the relative degree of sex reversal. As indicated, they ranged all the way from no reversal to complete female expression in the tassel (figs. 1 *a*, 2 *a–g*, 3 *a*). A few records are as follows: 1 tassel had 1 silk, 1 had 2, 2 had 3, 2 had 4, 2 had 5, 2 had 6, 1 had 9, 1 had 10, 2 had 15, 1 had 16, 1 had 18, 1 had 19, 2 had 20, 1 had 23, 1 had 28, 1 had 33, 1 had 42, 1 had 50, 1 had 53, 1 had 88, and 1 had 112. The reversal is usually complete and produces a good grain, even when only a single flower is affected and a single silk developed in the entire tassel. These silks may be in any position in the tassel or its branches, from the bottom to the top. Thus a well developed tassel having 5 silks had 2 of them near the base, 2 above the middle, and 1 within half an inch of the tip. More commonly the sex reversal is mainly at or near the base of the inflorescence.

A summary of all the plantings is shown in table I. It is evident that with uniform and favorable conditions the degree of sex reversal varies with the relative length of daylight, very much as the writer found true for hemp (**6, 9**).

Although the vegetative period of development seems little changed by the short photoperiodicity, the reduction of the number

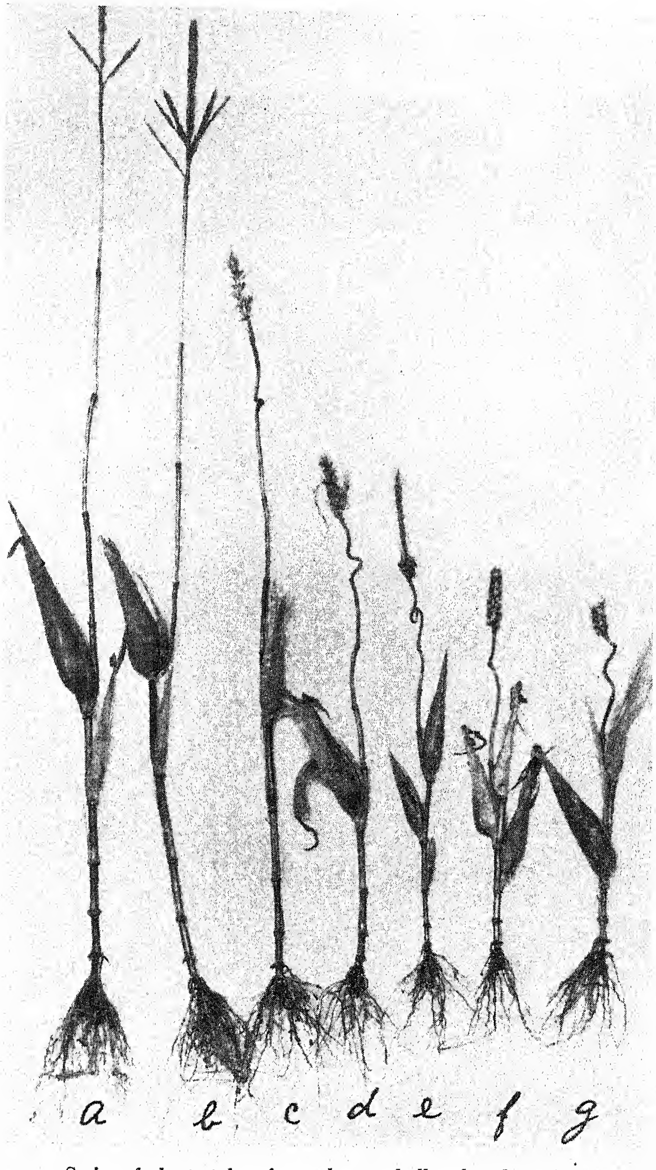

FIG. 2.—Series of plants taken from plot on shallow benches, showing gradation from normal monoecious condition to rather extreme carpellate development; leaves removed to show flexuous condition of the partially reversed plants: *a*, *b*, normal monoecious condition; *c–g*, various degrees of sex reversal in tassel.

of nodes and leaves is very marked. One of the most striking changes is produced in the main stem. With the change of functional gradient and in the extreme conditions of reversal, femaleness is mostly determined some distance below the tassel. In the most extreme cases, the secondary female state was induced in the second or third nodes from the base of the stem (figs. 1 *b*, 3 *a*). Since the

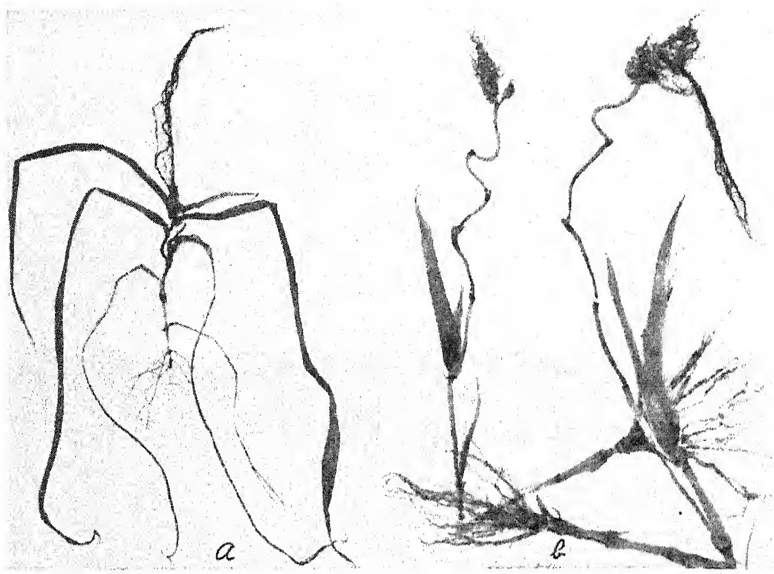

FIG. 3.—*a*, extreme reversal condition, showing complete female expression; *b*, two plants with leaves removed, both showing decided sex reversal in tassels (but still somewhat branched); femaleness determined in main shoot just above uppermost ear-producing node, consequently terminal stems decidedly flexuous.

secondary female state which develops in the incepts of the lateral or ear buds throws the leaf sheaths into the husk condition, and also causes a flexuous development of the shank or ear stem, especially when this is somewhat elongated, one would expect a somewhat similar action in case femaleness is developed in the main stem. In the more extreme cases the sheaths are decidedly influenced, showing considerable husk characteristics, and the stem becomes remarkably flexed, in some cases even developing double loops when the internodes are long (fig. 2 *e*). The flexuous condition is well shown

in figs. 1 *b*, 2 *c–g*, and 3 *b*. Fig. 2 *a* and *b* show the normal straight stems of normal monoecious plants in which no sex reversal has taken place. Fig. 2 *c* has a slight double kink in the first node below the tassel which showed a partial sex reversal. In figs. 1 *b* and 3 *a* the flexuous condition is shown in one or more nodes below the first ear bud. These stems are at first perfectly straight, but as lengthening of the internodes proceeds the one-sided development becomes very

TABLE I

SUMMARY OF PLANTINGS SHOWING PERCENTAGE OF REVERSAL IN TASSEL

Date of planting	Total no. of plants	Reversed tassels	Not reversed	Percentage of reversal	Vegetative period (days)
1925–26					
December 14	28	21	7	75	....
December 17*	55	44	11	80	....
1926–27					
November 1†	20	20	0	100	75
November 2	17	13	4	76.5	....
November 16	11	8	3	72.5	....
December 17	9	5	4	55.5	....
February 14†	25	3	22	12	78
March 15	27	1	26	3.6	72

* Variety in this plot was Country Gentleman sweet corn.

† Planted in deep soil in tank.

prominent, forcing the sheaths apart (fig. 1 *b*), and often causing the tip of the plant to be bent toward the ground when the support given by the sheaths is lost. These extreme plants with their flexuous stems and terminal silks present a very bizarre appearance when compared with corn plants with the normal hereditary expression. Such plants can now be produced easily at any time for demonstration purposes with the proper environmental control.

The tassels showing sex reversal are usually only slightly branched. This is the usual effect of the presence of the female state, although the tassels without sex reversal developed in a shorter light period than the normal are also only slightly branched and occasionally not at all.

The reversal of the tassel to the carpellate condition in the two varieties of sweet corn tested is to be regarded as an ecological fluctuation depending on a complex of environmental factors. With the

proper moisture, nutrient supply, temperature, and dimness of light, the amount of reversal, both in respect to the population as a whole and the degree to which it is expressed in any individual, is proportional to the shortness of the daily illumination period. Since intensity of illumination seems to have a decided influence, proper spacing is also important if one is attempting to obtain uniform reversal ratios. Perhaps the percentage of reversal will also be found proportional to the degree of light of low intensity, if the photoperiodicity is kept constant.

Summary

1. Narrow-grain Evergreen sweet corn is decidedly influenced in its sexual expression by the length of the daily illumination period, and the amount and degree of reversal to femaleness in the tassel is subject to experimental control. Limited experiments with Country Gentleman sweet corn indicate that the same condition is present in that variety.

2. Corn planted on November 1 in the greenhouse will, with proper substratum and heat, show 100 per cent of the individuals with some degree of female expression in the tassel, while that planted in the spring or summer will normally show only pure staminate tassels.

3. Corn planted either before or after this date, under similar conditions, will show a ratio of sex reversal inversely proportional to the length of the daylight. With the approach of equal day and night periods of 12 hours each, little or no reversal will occur in the tassel.

4. The time and point of female sex determination can be thrown back to the second or third leaf nodes from the base of the plant.

5. Whenever the female state is established in the main stem, either above or below the point of lateral ear development, the internodes become decidedly flexuous, and in extreme cases they are even thrown into loops.

6. Any plot of corn, grown in the greenhouse, can be so planted and developed that the individuals of the population will show every gradation, from the normal monoecious type, with pure carpellate-

ness expressed in the side branches and pure staminateness in the terminal inflorescence, to an absolutely pure female condition, all the flowers of the terminal inflorescence as well as those of the lateral ears being purely carpellate.

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CURRENT LITERATURE

BOOK REVIEWS

Flowering plants

The second volume of RENDLE'S[1] presentation of the classification of flowering plants has appeared twenty years after the publication of the first volume. The author states that "increasing official and extra-official duties and responsibilities have allowed insufficient leisure for the continuous effort required." This delay has resulted in the advantage of his being able to include the recent advances in our knowledge of dicotyledons and their relationships.

The general arrangement follows that of ENGLER'S *Syllabus der Pflanzenfamilien*, and the nomenclature is that adopted by the Vienna Congress of 1905. Three grades are recognized in the phylogeny of dicotyledons, Monochlamydeae, Dialypetalae, and Sympetalae. The author does not claim that the sequence is strictly phylogenetic. He states that "various attempts have been made to construct a phylogenetic system of angiosperms, but the results are not convincing, bear no suggestion of permanence, and bristle with difficulties for the student."

The three grades used correspond to grades of differentiation in the floral structure. The Monochlamydeae include the orders with a comparatively simple type of flower. Several of them represent very isolated groups. This grade extends from the Salicales to the Centrospermae, in the latter order the higher type of floral structure existing parallel with the simple monochlamydeous type. In the Dialypetalae, extending from the Ranales to the Umbelliflorae, the orders are arranged in ascending sequence, from indefinite to definite numbers of floral members, from spiral to cyclic arrangement, from hypogyny to epigyny, and from regularity to zygomorphy. The Sympetalae represent still higher grades of floral complexity, extending from the Ericales to the Campanulales. The evidence is that this culminating group of the plant kingdom has arisen from various groups of the Dialypetalae.

Each family is presented in great detail and well illustrated. Not only are the distinctive characters given, but also a full account of suggestive relationships, geographical distribution, life habits, cultivation, and uses. In short, the purpose seems to be to present every known aspect of the family, so that one may become fully acquainted with it in its setting in the plant kingdom. In a

[1] RENDLE, A. B., The classification of flowering plants. Vol. II. Dicotyledons. 8vo. pp. xix+636. *figs. 279.* 1925. London: Cambridge University Press; New York: Macmillan Co.

book which is such a thesaurus of information it is impossible for a brief review to select any details. The volume will certainly prove to be an invaluable help, not only to students of classification, but also to all botanists.—J. M. C.

Genetics in relation to agriculture

The second edition of the volume by BABCOCK and CLAUSEN,[2] which the authors promised us several years ago, has just appeared. This delay in publication has been well worth while, permitting the inclusion of numerous very significant investigations of the past few years. So conscientiously have the authors attempted to keep pace with this most rapidly moving of all subjects, that the present text bears only a superficial resemblance to its predecessor of nine years ago.

In total pages the two editions are practically the same. There has been, however, a considerable enlargement of the material on fundamental genetics (Part I), with a compensating reduction of the practical applications to plant and animal breeding (Parts II and III).

The fourteen chapters which comprised Part I of the earlier edition are replaced by twenty-seven shorter chapters, a device which makes the book much more readable, particularly for the undergraduate. The sequence of subject matter in this part is thoroughly recast. A much more substantial foundation is laid by the thirty-one pages on "Physical basis of heredity" than the eleven pages which were there before. Following this, chapters are grouped under the two general headings of Heredity and Variation, in the sequence indicated, which is a worth while reversal of the sequence in the earlier volume.

Of the twelve chapters on Heredity, three are very largely new, comprising two short chapters on Lethal factors and Sex limited characters, and a long one on Organization of the linkage groups. This last chapter, like the handbook for an automobile, makes the machine more than a mere convenience, and actually tells us how to take it apart and put it together again.

The general section on Variation contains a much more refined analysis of mutation than before. Instead of one chapter on the subject there are now five. Factor mutations (Chapter 22), Chromosomal variation (Chapter 24), and Oenothera investigations (Chapter 26) might have been expected. Chapters 23 and 25, however, are a delightful surprise. The former, on Parallel variation, devotes thirteen pages to the very suggestive problem of factor mutations of independent origin in related species. The authors have wisely set apart from Chromosomal variation a special subject which merits an identity of its own. In Chapter 25, under the title Sectional variation, they discuss the instances where a portion of a chromosome has become lost or translocated.

After reading the text, one is not surprised to find thirty-five pages devoted to the list of literature (approximately a thousand titles). The general biological

[2] BABCOCK, E. B., and CLAUSEN, R. E., Genetics in relation to agriculture. pp. xiv+673. *pls. 4. figs. 203.* New York: McGraw-Hill Book Co. 1927. $5.00.

public, as well as the authors, are to be congratulated upon the appearance of this volume.—M. C. COULTER.

Fungi

GWYNNE-VAUGHAN and BARNES[3] have published a much needed textbook, presenting the fungi to students rather than to investigators. It includes all the fungi, so that the student may obtain a general perspective of this very complex assemblage. Pathology is not included, since the authors state that they "are concerned primarily with the fungus, and only secondarily with its effect on other organisms."

The organization of the textbook is suggestive. It begins with a general account of the structure of fungi, including the Myxomycetes and Plasmidiophoraceae, "forms resembling fungi." The physiology of fungi is presented under the following headings: saprophytism, parasitism, symbiosis, specialization, and reaction to stimuli. Twelve major groups are then presented, from Phycomycetes to Autobasidiomycetes, followed by an account of the Fungi Imperfecti. These groups are fully described and well illustrated. The descriptions are not in technical style, but in language that will be understood by the student. The book closes with a very effective description of mycological technique, so that the student may know how fungi are investigated.

This book will prove to be a very effective introduction to fungi for students, the presentation being so well adapted to their needs that they will not only be instructed, but also interested.—J. M. C.

Soil mineralogy

A useful little handbook on soil mineralogy has been prepared by BURT,[4] and those who want a more intimate knowledge of the species of minerals found in their local soils will find it a valuable aid. The first section gives in three brief chapters some general information regarding the physical properties of soil minerals, such as color, streak, luster, tenacity, hardness, cleavage, fracture, specific gravity, fusibility, form, and structure; the elements actually occurring in soils, including twenty-one of the more commonly found elements; and the processes of weathering, which are considered under the headings hydration, carbonation, oxidation, hydrolysis, reduction, sulphation, and chloridation.

The second part presents a tabulated view of the analysis of minerals by blowpipe methods. The key suffices for the determination of somewhat more than a hundred of the commoner minerals of the soil. The third section describes about sixty-five minerals in ten classes, halides, sulphides, oxides, hydroxides and hydrous oxides, silicates, carbonates, sulphates, phosphates, borates, and nitrates, and metaferrite and metatitanite. The fourth part consists of four

[3] GWYNNE-VAUGHAN, H. C. I., and BARNES, B., The structure and development of the fungi. London: Cambridge University Press. 8vo. pp. xvi+384. *figs. 285.* 1927.

[4] BURT, F. A., Soil mineralogy. 8vo. pp. viii+81. New York: Van Nostrand. 1927.

tables showing the general occurrence of soil minerals, their relative weathering resistance, the volume changes involved in weathering changes, and the physical properties of twenty-one of the commoner soil constituents.

The brevity and conciseness of the book appeal to the student. In many cases one would want to consult the larger works after having exhausted the information obtainable from use of this volume, but it should be a welcome aid to everyone who uses soils in connection with the investigation of plant life.—C. A. SHULL.

Hydrogen ion concentration

A recent translation[5] of MICHAELIS's *Wasserstoffionenkonzentration* needs no description for American students of plant physiology. The earlier German editions have been used very widely in this country, but it will be more widely available and still more widely used in this translation by Professor PERLZWEIG.

Some of the more recent advances in this field have been added by MICHAELIS, so that it is more valuable even than the second German edition. These additions concern the newer contributions to the activity theory of ionization, recent modification of the theories of dissociation of ampholytes, and the theories of oxidation-reduction potentials, including use of the quinhydrone electrode.

Students will find it very worth while to study the volume carefully. The first five chapters lay down the general principles of physical chemistry now so much needed by biological investigators, and the last five deal with ions as sources of electrical potential differences. An important service has been rendered in the translation, which has been exceedingly well done. In its appearance as a book, it lives up to the excellent traditions of the publishers.—C. A. SHULL.

Flora of Jamaica

In continuation of their *Flora of Jamaica*, FAWCETT and RENDLE[6] have published a volume describing the dicotyledons from Buxaceae to Umbelliferae, completing what they call the "free-petaled" dicotyledons. The volume is thoroughly organized, with analytical keys, full descriptions, excellent illustrations, complete synonymy and literature, and geographical distribution. It includes 42 families, represented by 158 genera and 467 species. In the main the genera are represented by very few species, no fewer than 80 of them being monotypic, so far as Jamaica is concerned. Much the largest families are the Malvaceae (69 species), Melastomaceae (69 species), and Myrtaceae (60 species). The largest genus is *Eugenia* (Myrtaceae) with 37 species, the next in size being *Miconia* (Milastomaceae) and *Sida* (Malvaceae), each with 19 species.

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[5] MICHAELIS, LEONOR, Hydrogen ion concentration, its significance in the biological sciences and methods for its determinations. Transl. by WM. A. PERLZWEIG. 8vo. pp. xvi+299. Baltimore: Williams and Wilkins.

[6] FAWCETT, W., and RENDLE, A. B., Flora of Jamaica. Vol. V, Part III. 8vo. pp. xxviii+453. *figs. 156*. British Museum Publ. 1926.

Most of the genera are represented by only one or two species. The contrasts with the flora of the United States are often very striking. For example, the Umbelliferae are represented in Jamaica by only 6 species, representing 3 genera. Many such contrasts occur, and are very suggestive to students of geographical distribution.—J. M. C.

Fungi of Middle Europe

Numbers 3 and 4 of volume I of *Fungi of Middle Europe*[7] have made their appearance. The general scheme of this new work on fungi has been reviewed previously.[8] Number 3 carries a discussion of *Boletus rhodoxanthus*, *B. impolitus*, *B. pseudosulphurens*, and *B. pulverulentes*, with two colored plates of the last two species, and two plates in black and white illustrating habit and structural features of these and of *B. rimosus*, *B. parasiticus*, and *B. regius*. Part IV consists of a discussion of *B. rimosus* and *B. erythropus*, and of two colored plates of these organisms.—G. K. K. LINK.

Zoocecidia of Netherlands East Indies

A comprehensive work has been published on the animal galls of the Dutch East Indies.[9] The first nine chapters are devoted to general topics, such as collection of gall producers, geographical distribution of galls, and phylogenetic considerations (H. H. KARNY). Chapter X, which comprises the major part of the text, gives descriptions of galls, beginning with those of the Hymenophyllaceae and ending with those of the Compositae. There are seven plates (four in color), and bibliographical, host, and pathogen indices.—G. K. K. LINK.

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[7] Die Pilze Mitteleuropas, under the editorship of KNIEP, H. (Berlin), CLAUSSEN, P. (Marburg), and BASZ, J. (Stuttgart). Leipzig: W. KLINGHARDT. Band I. No. 1. 1926. M. 4.00 per number.

[8] BOT. GAZ. 83:213. 1927.

[9] DOCTERS VAN LEEUWEN-REIJNVAAN, MRS. J., and DOCTERS VAN LEEUWEN, W. M., The zoocecidia of the Netherlands East Indies. pp. 601. *pls. 7. figs. 1088.* Batavia: Drukkerij de Unie. 1926.

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GENERAL INDEX

Classified entries will be found under Contributors and Reviewers. New names and names of new genera, species, and varieties are printed in **bold-face** type; synonyms in *italics*.

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